Exhibit 2

Complaint Exhibit 2 – Onpattro Infringement of U.S. Patent No. 8,895,717

U.S. Patent No. 8,895,717	Patisiran/Onpattro
[Claim 2, Preamble] A pharmaceutical composition comprising:	**To the extent that the preamble is limiting, Onpattro is a pharmaceutical composition.** (see below)

Table 1: Quantitative Composition of Patisiran Drug Product

Component	Content per Volume (mg/mL)	Content per Vial (mg)	Function	Quality Standard
Patisiran drug substance (patisiran sodium)	2.0 patisiran (equivalent to 2.1 patisiran sodium)	Patisiran 10.0 (equivalent to 10.5 patisiran sodium)	Active ingredient	Manufacturer's specifications
DLin-MC3-DMA	13.0	65.0		Manufacturer's specifications
PEG_{2000}-C-DMG	1.6	8.0		Manufacturer's specifications
DSPC	3.3	16.5		Manufacturer's specifications
Cholesterol	6.2	31.0		USP/NF, Ph. Eur., JP
PBS[a]				
Sodium phosphate, dibasic, heptahydrate	2.3	11.7		USP, Ph. Eur
Potassium phosphate, monobasic, anhydrous	0.2	0.9		NF
Sodium chloride	8.8	44.0		USP, Ph. Eur
Water for injection	qs	qs		USP, Ph. Eur

a values for content per volume have been rounded to two significant figures; content per vial is calculated using non-rounded values

Abbreviations: JP=Japanese Pharmacopoeia; LPN=lipid nanoparticles; NF=National Formulary; PBS=phosphate buffered saline; Ph. Eur =European Pharmacopoeia; quantum sufficient; USP=United States Pharmacopoeia

Sodium content is 3.99 mg/mL and 20.0 mg/vial

Source 1 at 29-30

See also evidence and explanation *infra* at Claim 2, Element (b)(6)

To the extent this limitation is not met literally, it is met under the doctrine of equivalents.

U.S. Patent No. 8,895,717	Patisiran/Onpattro
[Claim 2, Element (a)(1)] (a) a short inhibitory ribonucleic acid (siRNA) component comprising one or more siRNA,	**Onpattro comprises an siRNA.**

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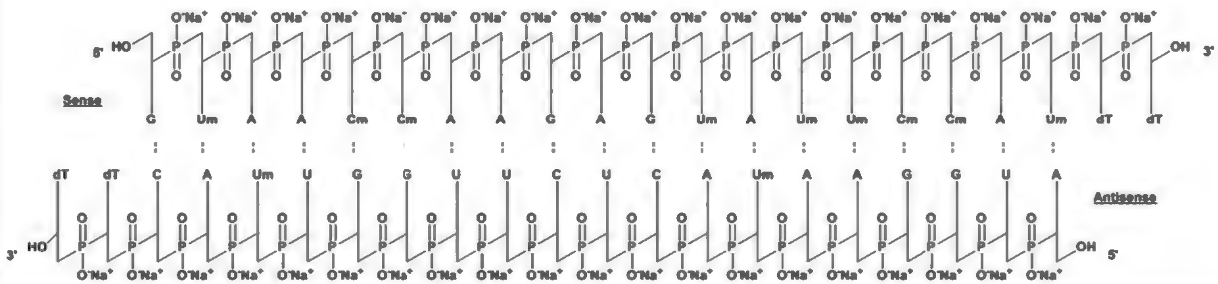
	Patisiran is a synthetic small interfering ribonucleic acid (siRNA) formulated as lipid nanoparticle (LNPs) containing 2 mg/mL patisiran and lipid excipients in phosphate buffered saline for slow IV infusion. It is indicated for treatment of hereditary transthyretin (TTR) mediated amyloidopathy (hATTR), a rare disease with a median survival time of 4.7 years following onset of symptoms. The proposed dosage is 0.3 mg/kg administered every three weeks. Patisiran was granted orphan drug, fast track, and breakthrough therapy designations, in 2012, 2013, and 2017, respectively. Source 1 at 10 **11 DESCRIPTION** ONPATTRO contains patisiran, a double-stranded small interfering ribonucleic acid (siRNA), formulated as a lipid complex for delivery to hepatocytes. Patisiran specifically binds to a genetically conserved sequence in the 3' untranslated region (3'UTR) of mutant and wild-type transthyretin (TTR) messenger RNA (mRNA). The structural formula is:  Source 2 at 8 To the extent this limitation is not met literally, it is met under the doctrine of equivalents.
[Claim 2, Element (a)(2)] wherein the siRNA has a backbone moiety that is negatively charged; and	**Onpattro's siRNA has a backbone moiety that is negatively charged.** Source 2 at 8 (reproduced at Claim 2 Element (a)(1)) To the extent this limitation is not met literally, it is met under the doctrine of equivalents.
[Claim 2, Element (b)(1)] (b) a lipid component comprising	**Onpattro has a lipid component.**

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	Patisiran lipid complex injection is a sterile, preservative- free, white to off-white, opalescent, homogeneous liquid for intravenous infusion. It is supplied as a 5-mL liquid containing 10 mg patisiran (2 mg/mL) in a 10-mL, single dose, Type [redacted] (b) (4) glass vial. In the product formulation, the patisiran siRNA is encapsulated in novel lipid nanoparticles (LNPs). The nanoparticles are suspended in a PBS buffer. The lipid components of the formulation include two novel excipients, DLin-MC3-DMA[1] ([redacted] (b) (4) and PEG2000-C-DMG[2] ([redacted] (b) (4) DSPC[3] [redacted] (b) (4) and cholesterol [redacted] (b) (4) Source 1 at 12 4.2. **Product Quality** Patisiran (ALN-TTR02; patisiran-LNP) is a ribonucleic acid (RNA) interference (RNAi) therapeutic product comprised of 2 mg/mL patisiran drug substance (ALN-18328) and lipid excipients DLin-MC3-DMA, DSPC, cholesterol, and PEG2000-C-DMG as lipid nanoparticles (LNPs) in isotonic phosphate buffered saline. Source 5 at 65 To the extent this limitation is not met literally, it is met under the doctrine of equivalents.
[Claim 2, Element (b)(2)] a phospholipid component consisting of one or more neutral phospholipids selected from the	**Onpattro has a phospholipid component consisting of at least a phosphatidylcholine.**

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group consisting of a phosphatidylcholine or a phosphatidylethanolamine phospholipid, wherein the phospholipid is not a lysophosphatidylcholine or lysophosphatidylethanolamine and	ONPATTRO is supplied as a sterile, preservative-free, white to off-white, opalescent, homogeneous solution for intravenous infusion in a single-dose glass vial. Each 1 mL of solution contains 2 mg of patisiran (equivalent to 2.1 mg of patisiran sodium). Each 1 mL also contains 6.2 mg cholesterol USP, 13.0 mg (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate (DLin-MC3-DMA), 3.3 mg 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1.6 mg α-(3'-{[1,2-di(myristyloxy)propanoxy] carbonylamino}propyl)-ω-methoxy, polyoxyethylene (PEG_{2000}-C-DMG), 0.2 mg potassium phosphate monobasic anhydrous NF, 8.8 mg sodium chloride USP, 2.3 mg sodium phosphate dibasic heptahydrate USP, and Water for Injection USP. The pH is ~7.0. Source 2 at 8-9 **Pharmacology** *Primary Pharmacology* The siRNA component of ALN-TTR02 is encapsulated in a LNP composed of two novel excipients, DLin-MC3-DMA and PEG_{2000}-C-DMG, as well cholesterol and the synthetic phosphatidylcholine 1,2-distearolyl-sn-Glycerol-3-phosphocholine (DSPC). According to the sponsor, upon IV administration of ALN-TTR02, PEG_{2000}-C-DMG dissociates from the drug product, allowing endogenous apolipoprotein E (ApoE) to bind with the LNP and facilitate low density lipoprotein receptor-mediated uptake by hepatocytes. Following hepatic uptake, the LNP becomes positively charged and disintegrates in the hepatocyte cytosol, releasing the siRNA that silences WT and mutant TTR mRNA. ALN-18328, the siRNA component of ALN-TTR02, is complimentary to a sequence of TTR mRNA conserved in monkeys and humans, but not rodents or rabbits. Studies with ALN-TTR01, which according to the sponsor is ALN-18328 formulated with an earlier and less-potent LNP composition, indicated an ED_{50} of 1 mg/kg for TTR mRNA silencing in cynomolgus monkeys, and reductions in serum TTR protein of up to 90 and 30% on postdose days 14 and 28, respectively, after administration of a single IV infusion of 0.3 mg/kg ALN-TTR01. Additionally, a study using a transgenic mouse model of TTR-amyloidosis (H129-hTTR V30M/Hsf-1 KO) indicated significant reductions in TTR protein immunoreactivity in esophagus, colon, stomach, sciatic nerve, and dorsal ganglion following six, twice-weekly IV bolus injections of 3 mg/kg ALN-TTR01. Using ALN-TTR02, single IV dosing up to 0.3 mg/kg in cynomolgus monkeys resulted in a 94% decrease in hepatic TTR mRNA and 80 and 70% decreases in serum TTR protein on Days 14 and 28, respectively. IV infusion of 0.15, 0.2, 0.25, or 0.3 mg/kg ALN-TTR02 monthly or every 3 weeks for 7 or 8 doses resulted in dose-dependent decreases of up to 95% in serum TTR in cynomolgus monkeys, with greater suppression occurring after each dose until the third or fourth dose. Source 5 at 358

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<table>
<tr>
<td></td>
<td>
SUMMARY OF ANALYTICAL RESULTS AND INTERPRETATIONS
Onpattro (Patisiran) Lipid Complex Injection (S2) was screened for neutral phospholipids by LC/MS. Excluding DSPC and lyso forms of DSPC (18:0 Lyso PC and 2-18:0 Lyso PC), the next two major neutral phospholipids found in S2 were identified as 18:0-20:0 PC and PSPC or 16:0-18:0 PC. The results are outlined below.
<table>
<tr><th>COMPOUND NAME</th><th>CAS NUMBER</th><th>MOLECULAR FORMULA</th><th>MASS (M/Z)</th><th>%AREA RELATIVE TO DSPC</th></tr>
<tr><td>1-stearoyl-2-arachidoyl-sn-glycerol-3-phosphocholine (18:0-20:0 PC)</td><td>61574-14-9</td><td>$C_{46}H_{92}NO_8P$</td><td>818.6633</td><td>7.14</td></tr>
<tr><td>1-palmitoyl-2-stearoyl-sn-glycerol-3-phosphocholine (PSPC, 16:0-18:0 PC)</td><td>59403-51-9</td><td>$C_{42}H_{84}NO_8P$</td><td>762.6010</td><td>2.87</td></tr>
</table>
Source 3 at 2

CONCLUSIONS
Onpattro (Patisiran) Lipid Complex Injection (S2, 10 mg/5 mL) was screened for neutral phospholipids by LC/MS. In addition to DSPC and Lyso PC, the next two major neutral phospholipids were identified as PSPC (16:0-18:0 PC, CAS# 59403-51-9) and 18:0-20:0 PC (CAS# 61574-10-5) with the structures shown in Figure 30 and Figure 31. The positive mode LC/MS data supports the assignment of the molecular formulas and the presence of the phosphocholine moiety in each structure. The negative mode LC/MS data supports the assignment of the fatty acids present in the neutral phospholipids based on the MS^2 fragment ions.

Source 3 at 17
To the extent this limitation is not met literally, it is met under the doctrine of equivalents.
</td>
</tr>
<tr>
<td>[Claim 2, Element (b)(3)]
further wherein the lipid component has a neutral charge;</td>
<td>Onpattro’s lipid component has a neutral charge.
Source 2 at 8-9 (reproduced at Claim 2, Element (b)(2))
To the extent this limitation is not met literally, it is met under the doctrine of equivalents.</td>
</tr>
<tr>
<td>[Claim 2, Element (b)(4)]
wherein the lipid component forms a liposome that encapsulate the siRNA such that greater than 90%</td>
<td>The lipid component forms liposomes that encapsulate the siRNA such that greater than 90% of the liposomes encapsulate siRNA.</td>
</tr>
</table>

of the liposomes encapsulate siRNA,	Following a single intravenous administration, systemic exposure to patisiran increases in a linear and dose-proportional manner over the range of 0.01 to 0.5 mg/kg. Greater than 95% of patisiran in the circulation is associated with the lipid complex. At the recommended dosing regimen of 0.3 mg/kg every 3 weeks, steady state is reached by 24 weeks of treatment. The estimated mean ± SD steady state peak concentrations (C_{max}), trough concentrations (C_{trough}), and area under the curve (AUC_{τ}) were 7.15 ± 2.14 μg/mL, 0.021 ± 0.044 μg/mL, and 184 ± 159 μg·h/mL, respectively. The accumulation of AUC_{τ} was 3.2-fold at steady state, compared to the first dose. In the placebo-controlled study, inter-patient variability in patisiran exposure did not result in differences in clinical efficacy (mNIS+7 change from baseline) or safety (adverse events, serious adverse events). Source 2 at 9 The development of LNP siRNA systems with high loading efficiencies, defined size and low surface charge satisfied the basic criteria for clinical potential; however, the potency of these systems for gene silencing in hepatocytes remained to be characterized and optimized. As the in vitro potency of an LNP nanomedicine rarely correlates with in vivo performance, we moved directly to an in vivo model to optimize gene silencing properties. LNPs containing siRNA against factor VII (FVII) were administered to mice Source 4 at 2 To the extent this limitation is not met literally, it is met under the doctrine of equivalents.
[Claim 2, Element (b)(5)] the siRNA is a double stranded nucleic acid of 18 to 100 nucleobases, and	**Onpattro's siRNA is a double stranded nucleic acid of 18 to 100 nucleobases.**

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	Patisiran is a double-stranded oligonucleotide comprising sense and antisense strands. The sense and antisense strands both contain 21 nucleotides. Nineteen nucleotides of the sense strand hybridize with the complementary 19 nucleotides of the antisense strand, forming 19 nucleotide base pairs, and leaving two 3'-terminal nucleotides on each strand as un-hybridized overhangs. Following IV infusion and targeted delivery of the lipid nanoparticles to hepatocytes in the liver, patisiran is released into the cytoplasm, where it can bind to and activate the RNA-induced silencing complex (RISC). The duplex then unwinds and the antisense strand binds to a genetically conserved sequence in the 3′ untranslated region of mutant and wild type TTR mRNA, resulting in degradation of the mRNA and reduction of wild type and mutant TTR protein synthesis. Source 1 at 10 Source 2 at 8 (reproduced at Claim 2, Element (a)(1)) To the extent this limitation is not met literally, it is met under the doctrine of equivalents.
[Claim 2, Element (b)(6)] the liposome encapsulated siRNA is comprised in a pharmaceutically acceptable carrier,	**Onpattro's liposome-encapsulated siRNA is comprised in a pharmaceutically acceptable carrier.** Source 1 at 12 (reproduced at Claim 2, Element (b)(1))

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	2.3 Preparation Instructions ONPATTRO must be filtered and diluted prior to intravenous infusion. The diluted solution for infusion should be prepared by a healthcare professional using aseptic technique as follows: • Remove ONPATTRO from the refrigerator and allow to warm to room temperature. Do not shake or vortex. • Inspect visually for particulate matter and discoloration. Do not use if discoloration or foreign particles are present. ONPATTRO is a white to off-white, opalescent, homogeneous solution. A white to off-white coating may be observed on the inner surface of the vial, typically at the liquid-headspace interface. Product quality is not impacted by presence of the white to off-white coating. • Calculate the required dose of ONPATTRO based on the recommended weight-based dosage *[see Dosage and Administration (2.1)]*. • Withdraw the entire contents of one or more vials into a single sterile syringe. • Filter ONPATTRO through a sterile 0.45 micron polyethersulfone (PES) syringe filter into a sterile container. • Withdraw the required volume of filtered ONPATTRO from the sterile container using a sterile syringe. • Dilute the required volume of filtered ONPATTRO into an infusion bag containing 0.9% Sodium Chloride Injection, USP for a total volume of 200 mL. Use infusion bags that are di(2-ethylhexyl)phthalate-free (DEHP-free). • Gently invert the bag to mix the solution. Do not shake. Do not mix or dilute with other drugs. • Discard any unused portion of ONPATTRO. • ONPATTRO does not contain preservatives. The diluted solution should be administered immediately after preparation. If not used immediately, store in the infusion bag at room temperature (up to 30°C [86°F]) for up to 16 hours (including infusion time). Do not freeze. Source 2 at 3

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	2.4 Infusion Instructions • Use a dedicated line with an infusion set containing a 1.2 micron polyethersulfone (PES) in-line infusion filter. Use infusion sets and lines that are DEHP-free. • Infuse the diluted solution of ONPATTRO intravenously, via an ambulatory infusion pump, over approximately 80 minutes, at an initial infusion rate of approximately 1 mL/min for the first 15 minutes, then increase to approximately 3 mL/min for the remainder of the infusion. The duration of infusion may be extended in the event of an IRR *[see Warnings and Precautions (5.1)]*. • Administer only through a free-flowing venous access line. Monitor the infusion site for possible infiltration during drug administration. Suspected extravasation should be managed according to local standard practice for non-vesicants. • Observe the patient during the infusion and, if clinically indicated, following the infusion *[see Warnings and Precautions (5.1)]*. • After completion of the infusion, flush the intravenous administration set with 0.9% Sodium Chloride Injection, USP to ensure that all ONPATTRO has been administered. *Id.* Source 5 at 65 (reproduced at Claim 2, Element (b)(1)) To the extent this limitation is not met literally, it is met under the doctrine of equivalents.
[Claim 2, Element (b)(7)] wherein the phospholipid component consists of two or more types of neutral phospholipids.	**The phospholipid component consists of two or more types of neutral phospholipids.** Source 3 at 2 (reproduced at Claim 2, Element (b)(2)) Source 3 at 17 (reproduced at Claim 2, Element (b)(2)) To the extent this limitation is not met literally, it is met under the doctrine of equivalents.
[Claim 10] The composition of claim 1, 2, 4, 6 or 5, wherein the composition further comprises cholesterol or polyethyleneglycol (PEG).	**Onpattro is the composition of claim 2 wherein the composition further comprises cholesterol or polyethyleneglycol (PEG).** Source 1 at 12 (reproduced at Claim 2, Element (b)(1)) Source 5 at 358 (reproduced at Claim 2, Element (b)(2)) To the extent this limitation is not met literally, it is met under the doctrine of equivalents.
[Claim 11] The composition of claim 1, 2, 4, 6 or 5, wherein the siRNA is 18 to 30 nucleobases.	**Onpattro is the composition of claim 2 wherein the siRNA is 18 to 30 nucleobases.** Source 1 at 10 (reproduced at Claim 2, Element (b)(5)) Source 2 at 8 (reproduced at Claim 2, Element (a)(1))

	To the extent this limitation is not met literally, it is met under the doctrine of equivalents.

Source 1 (Exhibit A to the Claim Chart): U.S. Food and Drug Administration, Center for Drug Evaluation and Research, *Application No. 210922Orig1s000 Product Quality Review(s)* (February 14, 2017)

Source 2 (Exhibit B to the Claim Chart): U.S. Food and Drug Administration-approved Onpattro Label (approved January 13, 2023)

Source 3 (Exhibit C to the Claim Chart): Identification of Neutral Phospholipids in Onpattro, EAG Laboratories

Source 4 (Exhibit D to the Claim Chart): Akinc, et al., *The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs*, Nature Nanotechnology (2019)

Source 5 (Exhibit E to the Claim Chart): U.S. Food and Drug Administration, Center for Drug Evaluation and Research, *Application No. 210922Orig1s000 Multi-Discipline Review* (August 10, 2018)

Exhibit A

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

210922Orig1s000

PRODUCT QUALITY REVIEW(S)

NDA 210922
ONPATTRO (patisiran) Lipid Complex Injection

Addendum to Drug Product Quality Review

Recommendation: Adequate

Drug Name/Dosage Form	Patisiran Lipid Complex Injection
Strength	10 mg/5 mL (2 mg/mL)
Route of Administration	Intravenous
Rx/OTC Dispensed	Rx
Applicant	Alnylam Pharmaceuticals

This is an addendum to NDA 210922 Drug Product Quality Review, dated 05-11-2018 by Mariappan Chelliah. This clarifies the shelf-life to be granted to the proposed commercial batches of the drug product.

Reviewer's Assessment: ***Adequate***

In the Drug Product quality review, dated 05-11-2018, based on the available stability data for the registration stability batches, this reviewer concluded that a shelf-life of 24 months may be granted to the drug product. However, in response to the Sponsor's email query dated 08-07-2018, the Agency seeks to clarify how the shelf-life is calculated for this product.

During the review cycle, the Sponsor updated the stability data for the registration batches and stated that the existing stability data support 24 months of storage for drug product from the date of fill (see page 47 of module 1.11.1, eCTD seq. 0016, dated 04-06-2018).

However, in a subsequent clarification (see page 18 of module 1.11.1, eCTD seq. 0020, dated 04-27-2018), the Sponsor stated the following:

"In the April 06 response to the Agency's information request (drug product question 7b) we stated that the existing stability data support 24 months of storage for drug product from the day of fill.

We would like to further clarify that the claimed drug product shelf life is to be dated from the date of bulk drug product production, in line with regulatory expectations that the date of drug product manufacture be defined as the time of drug product formulation.

The stability data as shown in the original NDA (Section 3.2.P.8.3 Stability Data), and the response to the Agency on April 7 demonstrate 24-month stability from the date of vial fill.

(b) (4)

[redacted]

Although the shelf-life is usually assigned from the compounding date, it is typically estimated from the stability data available from the fill date. However, because of the

(b) (4)

[redacted]

the available long-term real time stability data, and the statistical evaluation of the stability data, the Agency deems that the following shelf-life may be granted:

Shelf-life: 24 months from the date of (b) (4) vial filling and when stored at 2°C to 8°C. (b) (4)

Mariappan Chelliah	Digitally signed by Mariappan Chelliah Date: 8/08/2018 12:07:22PM GUID: 5399cb2c00032b7c21877aa0d4d5f794
Wendy Wilson- Lee	Digitally signed by Wendy Wilson- Lee Date: 8/08/2018 12:10:20PM GUID: 50816dbc000085595ca3284bbca465a8
Martha Heimann	Digitally signed by Martha Heimann Date: 8/08/2018 03:06:35PM GUID: 504f845f00000ed260627d268a8cdc9d

Recommendation: Approve

NDA 210922
Review 1

Drug Name/Dosage Form	Patisiran Lipid Complex Injection
Strength	10 mg/5 mL (2 mg/mL)
Route of Administration	Intravenous
Rx/OTC Dispensed	Rx
Applicant	Alnylam Pharmaceuticals
US agent, if applicable	N/A

Quality Review Team

DISCIPLINE	PRIMARY REVIEWER	SECONDARY REVIEWER
Drug Substance	Monica Cooper	Charles Jewell
Drug Product	Mariappan Chelliah	Wendy Wilson-Lee
Process	Erin Kim	Nallaperumal Chidambaram
Microbiology	Denise Miller	Bryan Riley
Facility	Christina Capacci-Daniel	Derek Smith
Biopharmaceutics	Banu Zolnik	Ta-Chen Wu
Regulatory Business Process Manager	Dahlia Walters	--
Application Technical Lead	Martha Heimann	--
Laboratory (OTR)	N/A	
ORA Lead	N/A	
Environmental	N/A	

SUBMISSION(S) REVIEWED	DOCUMENT DATE	DISCIPLINE(S) AFFECTED
SD-05, CMC module for rolling submission	11/15/2017	All
SD-07, Final NDA module	12/11/2017	Product, labeling
SD-10, Response to IR	03/05/2018	Biopharmaceutics, product
SD-12, Response to IR	03/08/2018	process
SD-16, Response to IR	04/06/2018	Drug substance, product
SD-17, Response to IR	04/09/2018	Microbiology
SD-20, Response to IR	04/27/2018	Biopharmaceutics, drug substance, product
SD-22, Response to IR	05/01/2018	Microbiology

Quality Review Data Sheet

1. RELATED/SUPPORTING DOCUMENTS

A. DMFs:

DMF #	Type	Holder	Item Referenced	Status	Date Review Completed	Comments
(b) (4) [redacted]	IV	[redacted]	(b) (4) [redacted]	Adequate	05/04/2018	
	V			Adequate	05/08/2018	
	V			Adequate [1]	02/03/2017	
	III			N/A [1]	N/A [1]	
	III			N/A [1]	N/A [1]	

[1] Adequate information in application or no changes to information since previous adequate reviews.

B. Other Documents: *IND, RLD, or sister applications*

DOCUMENT	APPLICATION NUMBER	DESCRIPTION
IND	117395	Development of patisiran for treatment of hereditary transthyretin mediated amyloidopathy (hATTR)

2. CONSULTS

None

Executive Summary

I. Recommendations and Conclusion on Approvability

The OPQ review team recommends APPROVAL of NDA 210922 for Onpattro™ (patisiran lipid complex injection) for intravenous infusion. A [redacted] (b) (4)-month retest date is granted for the drug substance when stored at [redacted] (b) (4)C in the proposed commercial container closure system, and a 24-month expiration dating period is granted for the drug product when stored refrigerated in the commercial packaging. ***The CMC post-marketing commitment (PMC) and post-approval quality agreements between OPQ and Alnylam listed below should be included in the action letter.***

<u>PMC</u>

Description: Development and validation of a new in vitro drug release method and setting of the drug release acceptance criteria for the finished drug product

Milestones: Submission of the Interim PMC Report within 6 months from NDA's action date (Type B WRO)
2/12/2019

Submission of the Final PMC Report within 12 months from NDA's action date (as Prior Approval CMC Supplement to the NDA)
8/12/2019

<u>Post-approval Quality Agreements</u>

We would like to remind you of the following post-approval quality agreements included in the amendment dated April 27, 2018 (SD-20).

- To provide the full-scale commercial manufacturing process data to support the in-process [redacted] (b) (4) by December 31, 2019.

- To validate the [redacted] (b) (4) method for the representative [redacted] (b) (4) impurities [[redacted] (b) (4)] for both strands of the patisiran drug substance and to provide the data to FDA by December 31, 2018. Per FDA's '*Guideline for Industry: Text on Validation of Analytical Procedures*,' quantitative test methods for impurities should include validation of specificity, linearity, precision (repeatability), intermediate precision, accuracy, range, and LOD/LOQ.

- To provide the validation data with respect to impurities for the drug product [redacted] (b) (4) methods post approval by December 31, 2018.

III. Summary of Quality Assessments

A. Product Overview

Proposed Indication(s) including Intended Patient Population	Treatment of adults with hereditary transthyretin-mediated amyloidosis
Duration of Treatment	Chronic
Maximum Daily Dose	0.3 mg/kg up to 30 mg maximum every three weeks.
Alternative Methods of Administration	None

Patisiran is a synthetic small interfering ribonucleic acid (siRNA) formulated as lipid nanoparticle (LNPs) containing 2 mg/mL patisiran and lipid excipients in phosphate buffered saline for slow IV infusion. It is indicated for treatment of hereditary transthyretin (TTR) mediated amyloidopathy (hATTR), a rare disease with a median survival time of 4.7 years following onset of symptoms. The proposed dosage is 0.3 mg/kg administered every three weeks. Patisiran was granted orphan drug, fast track, and breakthrough therapy designations, in 2012, 2013, and 2017, respectively.

TTR is a homotetrameric transport protein synthesized primarily in the liver, and is a carrier for retinol (vitamin A) and thyroxine. In individuals with a mutated copy of the gene encoding for TTR, the tetramer containing wild type and mutant TTR is less stable. Breakdown of TTR results in protein misfolding and aggregation to form amyloid fibrils that deposit in tissue, peripheral nervous system, and CNS. Depending on the location of the mutation, disease symptoms include polyneuropathy, cardiomyopathy, nephropathy, and gastrointestinal dysfunction. In the two principal phenotypes, the patients present with either polyneuropathy or cardiomyopathy.

Patisiran is a double-stranded oligonucleotide comprising sense and antisense strands. The sense and antisense strands both contain 21 nucleotides. Nineteen nucleotides of the sense strand hybridize with the complementary 19 nucleotides of the antisense strand, forming 19 nucleotide base pairs, and leaving two 3'-terminal nucleotides on each strand as un-hybridized overhangs. Following IV infusion and targeted delivery of the lipid nanoparticles to hepatocytes in the liver, patisiran is released into the cytoplasm, where it can bind to and activate the RNA-induced silencing complex (RISC). The duplex then unwinds and the antisense strand binds to a genetically conserved sequence in the 3′ untranslated region of mutant and wild type TTR mRNA, resulting in degradation of the mRNA and reduction of wild type and mutant TTR protein synthesis.

B. Quality Assessment Overview

Factors critical to the OPQ evaluation of the submission were the route of administration (intravenous), the absence of any approved products for treatment of a life-threatening rare disorder, and the complexity of the active ingredient and product formulation. Approval of

the application would be the first approval for a duplex siRNA, and the first approval for an siRNA lipid nanoparticle formulation.

Drug Substance

Patisiran is a chemically synthesized, double-stranded oligonucleotide comprising 21-residue sense and antisense strands hybridized across 19 nucleotide base pairs. The structure can be represented as shown in Figure 1, where hyphens represent the sodium form of a 3′ – 5′ phosphodiester linkage and the dotted lines represent the base pairs.

Figure 1: Structural Formula of Patisiran Drug Substance

```
Sense:          5'    G-Um-A-A-Cm-Cm-A-A-G-A-G-Um-A-Um-Um-Cm-Cm-A-Um-dT-dT    3'
                      ¦ ¦  ¦ ¦ ¦  ¦  ¦ ¦ ¦ ¦ ¦ ¦  ¦ ¦  ¦  ¦  ¦  ¦ ¦
Antisense:  3'  dT-dT-C-A-Um-U-G-G-U-U-C-U-C-A-Um-A-A-G-G-U-A    5'
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A, C, G, and U represent adenosine, cytidine, guanosine, and uridine r bonucleotide residues, respectively.
Cm and Um represent 2′-O-methylcytidine and 2′-O-methyluridine residues, respectively.
dT represents thymidine deoxyr bonucleotide residues.

The patisiran drug substance is a white to off-white powder. The double-stranded oligonucleotide is manufactured by (b) (4) [redacted]

Regulatory controls for patisiran drug substance include multiple orthogonal tests for identity and purity of the duplex siRNA. Identity of the duplex is confirmed by size exclusion chromatography (SE-HPLC UV) retention time, melting temperature, and molecular mass of the identity of the sense and antisense strands. Purity is determined (b) (4) [redacted]. The controls also include appropriate tests for appearance, sodium content, pH, water content, elemental impurities, residual solvents, endotoxins, and bioburden.

The drug substance is stored in a (b) (4) [redacted] The applicant's proposed (b) (4) [redacted] month retest date can be granted to the drug substance when stored at (b) (4) [redacted]°C in the proposed commercial container closure system.

Critical issues for the drug substance include: (b) (4) [redacted] The applicant has adequately addressed concerns identified during the review.

Drug Product

Patisiran lipid complex injection is a sterile, preservative- free, white to off-white, opalescent, homogeneous liquid for intravenous infusion. It is supplied as a 5-mL liquid containing 10 mg patisiran (2 mg/mL) in a 10-mL, single dose, Type [redacted] (b) (4) glass vial. In the product formulation, the patisiran siRNA is encapsulated in novel lipid nanoparticles (LNPs). The nanoparticles are suspended in a PBS buffer.

The lipid components of the formulation include two novel excipients, DLin-MC3-DMA[1] ([redacted] (b) (4) and PEG2000-C-DMG[2] ([redacted] (b) (4) DSPC[3] [redacted] (b) (4) and cholesterol. (b) (4) [redacted]

Patisiran lipid complex injection is manufactures in [redacted] (b) (4) drug product is compounded at the Alnylam Pharmaceuticals facility in Cambridge, MA. The drug substance is [redacted] (b) (4)

The regulatory specification for the drug product includes tests for physical parameters (i.e., siRNA encapsulation, particle size, and in vitro siRNA release) that are critical for protection of the siRNA in plasma and delivery to the liver. Assay, purity, and identity test methods for patisiran are similar to those for the bulk drug substance. The specification includes identity and assay for the individual lipid components, residual [redacted] (b) (4) (process aids), and all compendial testing (sterility, bacterial endotoxins, particulate matter, etc.) appropriate for a parenteral product.

---

[1] DLin-MC3-DMA: (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate

[2] PEG2000-C-DMG: (R)-2,3-bis(tetradecyloxy)propyl 1-(methoxypoly(ethyleneglycol)2000)propyl carbamate, or α-(3'-{[1,2-di(myristyloxy)propanoxy}carbonylamino}propyl)-ω-methoxy polyoxyethylene

[3] DSPC: 1,2-distearoyl-*sn*-glycero-3-phosphocholine

It is noted that the review team and the applicant have identified concerns related to the robustness of the siRNA in vitro release method. The current method is considered acceptable on an *interim basis*. The Applicant has agreed to develop a new validated and robust in vitro drug release method as part of Post Marketing Commitment within 12 months from the NDA's action date. If, however, the Application receives a Complete Response action based on deficiencies raised by other disciplines, then the recommendation/requirement to develop a new and optimal in vitro drug release method will be included in the CR letter as CR issues.

It is also noted that the acceptance criterion for product appearance [redacted] (b) (4) During the Phase 3 trial, the drug product was filtered through 0.2 µm sterile polyethersulfone (PES) filters. However, the entire maximum dose volume could not be filtered through a single 0.2 µm filter. Therefore, the applicant evaluated PES filters with larger pore sizes. Patisiran drug product filtered through 0.2 µm, 0.45 µm, [redacted] (b) (4) filters had comparable quality and complied with the product specification. Product labeling will specify use of a sterile 0 45 µm (PES) during dose preparation. The review team considers this an acceptable mitigation approach.

Patisiran lipid complex injection is packaged in a single-dose Type [redacted] (b) (4) glass vial with [redacted] (b) (4) stopper and an aluminum flip-off cap. Based on stability data provided in the application, a 24-month shelf life is granted for product stored at 2°C – 8°C.

Critical issues for the drug product include use of two novel synthetic lipid excipients (DLin-MC3-DMA and PEG2000-C-DMG), complexity of the manufacturing, sterilization and filling processes, stability of the drug product during manufacturing, shelf-life, and under in-use conditions, potential leachables from the vial and closure, and potential delamination of the glass vial. The applicant has adequately addressed concerns identified during the review.

Methods Verification

Verification of the drug substance and drug product [redacted] (b) (4) methods (identity, assay, and purity) and the [redacted] (b) (4) method (percentage of duplex form and total impurities) by the Division of Pharmaceutical Analysis (DPA) was requested; however, methods verification is not complete. As the methods verification process is ongoing, standard language regarding cooperation with methods verification should be included in the action letter.

Facilities

All facilities that will be involved in commercial manufacture and testing of patisiran and patisiran lipid complex injection are currently acceptable. The PMC to develop a more robust in vitro release method addresses some of the inspectional concerns noted at the drug product facility inspection and is in agreement with corrective actions proposed by the firm.

C. Special Product Quality Labeling Recommendations

The product should be stored at 2°C – 8°C and labeled "Do Not Freeze."
The product should be filtered through a sterile 0.45 µm polyethersulfone (PES) syringe filter during dose preparation.

D. Final Risk Assessment for Patisiran Lipid Complex Injection

From Initial Risk Identification			Review Assessment		
Attribute/ CQA	**Factors that can impact the CQA**	**Initial Risk Ranking**	**Risk Mitigation Approach**	**Final Risk Evaluation**	**Lifecycle Considerations/ Comments**
Appearance	• Formulation • Container closure • Raw materials • Process parameters • Scale • Equipment • Site	L	(b) (4) [redacted]	Adequate	
Assay (active), stability		L		Adequate	
Lipid component assay		L		Adequate	Currently 24-month expiry granted based on real time primary stability data
Lipid entrapment efficiency (bound vs. free drug)		H		Adequate	
In vitro release		H		Adequate	Post-marketing commitment
Particle size distribution		H		Adequate	
Sterility		H		Adequate	
Endotoxin, pyrogen		M		Adequate	
Fill volume/delivered volume		L		Adequate	

From Initial Risk Identification			Review Assessment		
Attribute/ CQA	**Factors that can impact the CQA**	**Initial Risk Ranking**	**Risk Mitigation Approach**	**Final Risk Evaluation**	**Lifecycle Considerations/ Comments**
Osmolality		**L**	(b) (4) [redacted]	Adequate	
pH (high)		**L**		Adequate	
pH (low)		**L**			
Particulate matter		**M**		Adequate	
Leachable/Extractables		**L**		Adequate	

Martha Heimann

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LABELING

I. Package Insert

The following assessment is based on the Applicant's labeling submissions in eCTD seq. 0007, dated 12-11-2017 and eCTD seq. 0011, dated 02-14-2018.

1. *Highlights of Prescribing Information*

Item	Information Provided in NDA
Product Title (Labeling Review Tool and 21 CFR 201.57(a)(2))	
Proprietary name and established name	**ONPATTRO (patisiran) lipid complex injection****
Dosage form, route of administration	lipid complex, injection**
Controlled drug substance symbol (if applicable)	NA
Dosage Forms and Strengths (Labeling Review Tool and 21 CFR 201.57(a)(8))	
Summary of the dosage form and strength	**Lipid Complex Injection: 10 mg/5 mL (2 mg/mL) in a single-dose vial

**Edit proposed by the Agency and yet to be accepted by the Sponsor (see the assessment section below for further information).

2. *Section 2 Dosage and Administration*

Item	Information Provided in NDA
(Refer to Labeling Review Tool and 21 CFR 201.57(c)(12))	
Special instructions for product preparation (e.g., reconstitution, mixing with food, diluting with compatible diluents)	The drug product must be filtered into a sterile container through a 0.45µm sterile syringe filter. The required volume, based on patient weight, is drawn and diluted into a saline bag to give the intravenous infusion solution.

3. *Section 3 Dosage Forms and Strengths*

Item	Information Provided in NDA
(Refer to Labeling Review Tool and 21 CFR 201.57(c)(4))	
Available dosage forms	Lipid Complex Injection**
Strengths: in metric system	10 mg/5 mL (2 mg/mL)
Active moiety expression of strength with equivalence statement (if applicable)	NA
A description of the identifying characteristics of the dosage forms, including shape, color, coating, scoring, and imprinting, when applicable.	…white to off-white, opalescent, homogeneous solution in a single-dose vial.

**Edit proposed by the Agency and yet to be accepted by the Sponsor (see the assessment section below for further information).

4. *Section 11 Description*

Item	Information Provided in NDA
(Refer to Labeling Review Tool and 21 CFR 201.57(c)(12), 21 CFR 201.100(b)(5)(iii), 21 CFR 314.94(a)(9)(iii), and 21 CFR 314.94(a)(9)(iv))	
Proprietary name and established name	Yes
Dosage form and route of administration	Yes
Active moiety expression of strength with equivalence statement (if applicable)	Yes
For parenteral, otic, and ophthalmic dosage forms, include the quantities of all inactive ingredients [see 21 CFR 201.100(b)(5)(iii), 21 CFR 314.94(a)(9)(iii), and 21 CFR 314.94(a)(9)(iv)], listed by USP/NF names (if any) in alphabetical order (USP <1091>)	Yes
Statement of being sterile (if applicable)	Yes
Pharmacological/ therapeutic class	Yes
Chemical name, structural formula, molecular weight	Yes
If radioactive, statement of important nuclear characteristics.	NA
Other important chemical or physical properties (such as pKa or pH)	pH ~7.0

5. ***Section 16 How Supplied/Storage and Handling***

Item	Information Provided in NDA
(Refer to Labeling Review Tool and 21 CFR 201.57(c)(17))	
Strength of dosage form	10 mg/5 mL (2 mg/mL)
Available units (e.g., bottles of 100 tablets)	Single vial per container
Identification of dosage forms, e.g., shape, color, coating, scoring, imprinting, NDC number	…white to off-white, opalescent, homogeneous solution for intravenous infusion…
Special handling (e.g., protect from light)	Do not freeze
Storage conditions	Store at 2°C to 8°C (36°F to 46°F).
Manufacturer/distributor name (21 CFR 201.1(h)(5))	Yes

Reviewer's Assessment of Package Insert: *Adequate*

Per labeling submitted in eCTD seq. 0011, the drug product is named as 'ONPATTRO (patisiran) injection, for intravenous use'. However, during the review cycle the Agency determined that the appropriate dosage form designation for this formulation is 'lipid complex injection". This reviewer has edited the product name in the prescribing information in the SharePoint to 'ONPATTRO (patisiran) lipid complex injection, for intravenous use'. The Agency will be recommending the Sponsor to use this name throughout the labeling.

II. Labels:

1. ***Carton Labels (from eCTD seq. 0011)***

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Item	Information provided in the container label	Information provided in the carton label(s)
Proprietary name, established name (font size and prominence (21 CFR 201.10(g)(2))	Onpattro (patisiran) injection	Onpattro (patisiran) lipid complex injection**
Dosage strength	10 mg/5 mL	10 mg/5 mL
Net contents	Missing – but acceptable for small container.	Single dose vial
"Rx only" displayed prominently on the main panel	Yes	Yes
NDC number (21 CFR 207.35(b)(3)(i))	Yes	Yes
Lot number and expiration date (21 CFR 201.17)	Yes	Yes
Storage conditions	Missing – but acceptable for small container.	Store refrigerated at 2°C to 8°C (36°F to 46°F). Do not freeze.
Bar code (21CFR 201.25)	Yes	Yes
Name of manufacturer/distributor	Manufactured for: Alnylam Pharmaceuticals, Inc. Cambridge, MA 02142	Manufactured for: Alnylam Pharmaceuticals, Inc. Cambridge, MA 02142 Manufactured by: Ajinomoto Althea, Inc. San Diego, CA 92121
And others, if space is available	--	--

**Edit proposed by the Agency and yet to be accepted by the Sponsor (see the assessment section below for further information).

Reviewer's Assessment of Labels: ***Adequate***

The carton and container labels meet the appropriate rules and regulations. As discussed above, the Agency will be recommending the Applicant to revise the name in the carton and container labels to 'Onpattro (patisiran) lipid complex injection'. Therefore, the labeling will likely change further.

List of Deficiencies: None

Overall Assessment and Recommendation: Adequate

Primary Drug Product Reviewer: Mariappan Chelliah (see below for date)

Secondary Reviewer: Wendy Wilson-Lee (see below for date)

Mariappan Chelliah

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Wendy Wilson- Lee

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BIOPHARMACEUTICS

Application No: NDA 210922

Drug Product Name / Strength: Onpattro (patisiran) Injection, 10 mg/5 mL (2 mg/mL)

Route of Administration: Injection; via intravenous infusion

Applicant Name: Alnylam Pharmaceuticals, Inc.

List of Submissions reviewed:
eCTD Seq.0005 (5) dated 11/15/2017 (Rolling NDA Part 1 of 2)
eCTD Seq.0010 (11) dated 03/05/2018 (In Response to Biopharmaceutics IR dated 02/20/2018)
eCTD Seq.0020 (20) dated 04/27/2018 (In Response to Biopharmaceutics IR dated 04/17/2018)

Background:
Alnylam Pharmaceuticals Inc is seeking approval for Onpattro (patisiran) injection as a treatment for adults with hereditary transthyretin-mediated amyloidosis via 505 (b)(1) of Federal Food, Drug and Cosmetic Act.

Review Summary:
This Biopharmaceutics Review evaluated the overall in vitro drug release data supporting the 1) proposed in vitro drug release method, 2) in vitro drug release acceptance criteria, as well as the need for 3) bridging of formulations, and 4) biowaiver request.

Based on the review of the provided information/data, the Division of Biopharmaceutics has the following conclusions and recommendations:

1) **In Vitro Drug Release Method and Acceptance Criteria**
The proposed in vitro drug release method and the proposed acceptance criteria are acceptable for batch release and stability testing of the finished product on an **interim basis.** The Applicant agreed to develop a validated and robust in vitro drug release method within 12 months from the NDA's action date as part of the Post Marketing Commitment. The details of the PMC are found in Appendix 1 of this review.

2) **Bridging the Formulations**
Bridging data are not necessary between the clinical and the proposed commercial formulations because there were no changes in the formulation or manufacturing process throughout the drug product development.

3) **Biowaiver Request**
Biowaiver Request is not submitted nor required. The Applicant characterized the pharmacokinetic profile of patisiran injection in the following studies: ALN-TTR02-001, ALN-TTR02-005 (Phase 1 SAD studies in healthy volunteers) and ALN-TTR02-002,

ALN TTR02-003 (Phase 2 multiple ascending dose studies in patients). These studies are reviewed by OCP reviewers (refer to OCP review in DARRTS dated 5/21/2018).

- ***OVERALL REVIEW RECOMMENDATION***

From the Biopharmaceutics perspective, NDA 210922 for Onpattro (patisiran) injection, 2 mg/mL is recommended for **APPROVAL.** The Applicant agreed to develop a new validated and robust in vitro drug release method as part of Post Marketing Commitment within 12 months from the NDA's action date. If, however, the Application receives a Complete Response action based on deficiencies raised by other disciplines, then the recommendation/requirement to develop a new and optimal in vitro drug release method will be included in the CR letter as CR issues.

- ***SIGNATURES***

Primary Biopharmaceutics Reviewer Name and Date:

Banu S. Zolnik, PhD 6/11/2018
Biopharmaceutics Reviewer
Division of Biopharmaceutics-Branch 1
Office of New Drug Products

Secondary Reviewer Name and Date:

Gerlie Gieser, PhD (for Ta-Chen Wu, Ph.D.) 6/11/2018
Acting Biopharmaceutics Lead
Division of Biopharmaceutics-Branch 1
Office of New Drug Products

BIOPHARMACEUTICS ASSESSMENT

➢ **DRUG SUBSTANCE:**

Patisiran is a double stranded small interfering RNA(siRNA) consisting of two single stranded RNA molecules (the sense and antisense strands).

➢ **DRUG PRODUCT:**

The proposed drug product is formulated as patisiran containing lipid nanoparticles in phosphate buffered saline. The pictorial representation of the nanoparticles, as provided by the Applicant, is shown in Figure 1.

(b) (4)

(b) (4)

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MICROBIOLOGY

Product Background: This is a parenteral drug product for the treatment of adults with hereditary transthyretin-mediated amyloidosis. This drug is to be administered by intravenous infusion via an ambulatory infusion pump over 80 minutes once every three weeks.

NDA: 210-922

Drug Product Name / Strength: Onpattro (patisiran) at 2 mg/mL, 5 mL fill in a 10 mL vial

Route of Administration: Intravenous

Applicant Name: Alnylam Pharmaceuticals, Inc.

Manufacturing Site:

Bulk Drug Product Manufacture

Alnylam Pharmaceuticals, Inc.
665 Concord Avenue
Cambridge MA 02138
FEI: 3013754451

Finish and Fill Manufacturer

Ajinomoto Althea, Inc.
11040 Roselle Street
San Diego CA 92121
FEI: 3004575449

Method of Sterilization: [redacted] (b) (4)

Review Recommendation: **Adequate**

Theme (ANDA only): **N/A**

Justification (ANDA only): N/A

Review Summary: **The information supporting the [redacted] (b) (4) filling of the vials at the contract manufacturing facility was reviewed under the facilities DMF (DMF [redacted] (b) (4)) and found to be acceptable. The product specific information supporting the sterility assurance of the proposed drug product is the subject of this review. There were two information requests (IR) sent in the course of this review for which the applicant submitted acceptable responses. The information provided was adequate and supports the [redacted] (b) (4) for the drug product.**

List Submissions Being Reviewed:
06 Nov 2017 Original NDA submission
09 Apr 2018 IR Response amendment
01 May 2018 IR Response amendment

Highlight Key Outstanding Issues from Last Cycle: NA

Remarks: NA

Concise Description Outstanding Issues Remaining: None

Supporting Documents:

DMF [redacted] (b) (4) [redacted] (b) (4) LOA dated 07/20/17 for the [redacted] (b) (4). The DMF is adequate per DMA review [redacted] (b) (4) dated 03 Feb 2017. The DMF has not added new information for the [redacted] (b) (4) since this review.

DMF [redacted] (b) (4) [redacted] (b) (4) *Facility in San Diego CA*, Ajinomoto Althea Inc. LOA 11/01/2017. DMA review ([redacted] (b) (4).docx) dated 05/08/18 was adequate.

List Number of Comparability Protocols (ANDA only): NA

S Drug Substance: drug substance is not sterile.

There is a bioburden and endotoxin release specification. The bioburden is NMT [redacted] (b) (4) cfu/gram and the endotoxin is NMT [redacted] (b) (4) EU/mg.

Note: The chemistry reviewer requested that DMA look at the excipients to determine if the ML and endotoxin limits are appropriate. There a 5 excipients, two are novel, one is not novel but does not have a USP monograph and two have a USP monograph; non are sterile.
The novel excipients are DLin-MC3-DMA and PEG_{2000}-C-DMG. The DLin-MC3-DMA is a lipid and the release specifications include bioburden (NMT [redacted] (b) (4) cfu/gr for both TAMC and TYMC), specified microorganism (absence of *Salmonella, E. coli, S. aureus* and *P. aeruginosa*) and has an endotoxin limit of NMT [redacted] (b) (4) EU/mg. The PEG_{2000}-C-DMG release specifications includes bioburden (NMT [redacted] (b) (4) cfu/g for both TAMC and TYMC) with an endotoxin limit of NMT [redacted] (b) (4) EU/mg.

A third excipient which is not novel is 1, 2-Distearoyl-sn-glucero-3-phosphocholine (DSPC). The release specifications for this excipient include bioburden (NMT [redacted] (b) (4) cfu/g for TAMC, NMT [redacted] (b) (4) cfu/g for TYMC, and absence of E. coli) with an endotoxin limit of NMT [redacted] (b) (4) EU/g.

The two USP monographed excipients are cholesterol and Phosphate Buffered Saline.

[redacted] (b) (4)

Reviewer's Assessment: ***Adequate;*** The information provided is acceptable. The bioburden and endotoxin of the bulk drug product is tested [redacted] (b) (4) .

P Drug Product

P.1 Description of the Composition of the Drug Product

- **Description of drug product** – patisiran sodium is a double-stranded small interfering ribonucleic acid formulated as a lipid nanoparticle in phosphate buffered saline. The particle size is [redacted] (b) (4) nm. The final drug product is a sterile, preservative free, white to off-white opalescent liquid.
- **Drug product composition** – the composition below was copied from 3.2.P.1 Table 1 of the submission.

Table 1: Quantitative Composition of Patisiran Drug Product

Component	Content per Volume (mg/mL)	Content per Vial (mg)	Function	Quality Standard
Patisiran drug substance (patisiran sodium)	2.0 patisiran (equivalent to 2.1 patisiran sodium)	Patisiran 10.0 (equivalent to 10.5 patisiran sodium)	Active ingredient	Manufacturer's specifications
DLin-MC3-DMA	13.0	65.0	(b) (4) [redacted]	Manufacturer's specifications
PEG_{2000}-C-DMG	1.6	8.0		Manufacturer's specifications
DSPC	3.3	16.5		Manufacturer's specifications
Cholesterol	6.2	31.0		USP/NF, Ph. Eur., JP
PBS[a]				
Sodium phosphate, dibasic, heptahydrate	2.3	11.7		USP, Ph. Eur.
Potassium phosphate, monobasic, anhydrous	0.2	0.9		NF
Sodium chloride	8.8	44.0		USP, Ph. Eur.
Water for injection	qs	qs		USP, Ph. Eur.

a values for content per volume have been rounded to two significant figures; content per vial is calculated using non-rounded values
Abbreviations: JP=Japanese Pharmacopeoia; LPN=lipid nanoparticles; NF=National Formulary; PBS=phosphate buffered saline; Ph. Eur.=European Pharmacopoeia; quantum sufficient; USP=United States Pharmacopoeia

Sodium content is 3.99 mg/mL and 20.0 mg/vial.

- **Description of container closure system –**
  - **Vial:** 10 mL Type (b) (4) [redacted] glass vial
  - **Stopper:** 20 mm gray [redacted] (b) (4)).

Reviewer's Assessment: ***Adequate,*** the information provided is a sufficient description for review.

P.2 Pharmaceutical Development

P.2.5 Microbiological Attributes

Container/Closure Integrity Testing (CCIT)

CCIT studies were provided using both a microbial ingress testing and helium leak testing.

(b) (4)

[redacted]

Reviewer's Assessment: ***Adequate;*** The CCIT studies provided support the integrity of the primary container closure system for the proposed drug product.
The shelf life container closure integrity is assessed in the stability program.

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Bryan Riley

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Martha Heimann

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Exhibit B

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use ONPATTRO® safely and effectively. See full prescribing information for ONPATTRO.

ONPATTRO (patisiran) lipid complex injection, for intravenous use
Initial U.S. Approval: 2018

----------------------INDICATIONS AND USAGE----------------------
ONPATTRO contains a transthyretin-directed small interfering RNA and is indicated for the treatment of the polyneuropathy of hereditary transthyretin-mediated amyloidosis in adults. (1)

-----------------DOSAGE AND ADMINISTRATION-----------------
- For patients weighing less than 100 kg, the recommended dosage is 0.3 mg/kg every 3 weeks by intravenous infusion. For patients weighing 100 kg or more, the recommended dosage is 30 mg (2.1)
- Premedicate with a corticosteroid, acetaminophen, and antihistamines (2.2)
- Filter and dilute prior to administration (2.3)
- Infuse over approximately 80 minutes (2.4)

----------------DOSAGE FORMS AND STRENGTHS----------------
Lipid Complex Injection: 10 mg/5 mL (2 mg/mL) in a single-dose vial (3)

------------------------CONTRAINDICATIONS------------------------
None (4)

------------------WARNINGS AND PRECAUTIONS-----------------
- Infusion-related reactions: Monitor for signs and symptoms during infusion. Slow or interrupt the infusion if clinically indicated. Discontinue the infusion if a serious or life-threatening infusion-related reaction occurs (5.1)
- Reduced serum vitamin A levels and recommended supplementation: Supplement with the recommended daily allowance of vitamin A. Refer to an ophthalmologist if ocular symptoms suggestive of vitamin A deficiency occur (5.2)

-------------------------ADVERSE REACTIONS------------------------
The most frequently reported adverse reactions (that occurred in at least 10% of ONPATTRO-treated patients and at least 3% more frequently than on placebo) were upper respiratory tract infections and infusion-related reactions (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Alnylam Pharmaceuticals at 1-877-256-9526 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 1/2023

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

ONPATTRO is indicated for the treatment of the polyneuropathy of hereditary transthyretin-mediated amyloidosis in adults.

2 DOSAGE AND ADMINISTRATION

2.1 Dosing Information

ONPATTRO should be administered by a healthcare professional.

ONPATTRO is administered via intravenous (IV) infusion. Dosing is based on actual body weight.

For patients weighing less than 100 kg, the recommended dosage is 0.3 mg/kg once every 3 weeks.

For patients weighing 100 kg or more, the recommended dosage is 30 mg once every 3 weeks.

Missed Dose

If a dose is missed, administer ONPATTRO as soon as possible.

- If ONPATTRO is administered within 3 days of the missed dose, continue dosing according to the patient's original schedule.
- If ONPATTRO is administered more than 3 days after the missed dose, continue dosing every 3 weeks thereafter.

2.2 Required Premedication

All patients should receive premedication prior to ONPATTRO administration to reduce the risk of infusion-related reactions (IRRs) *[see Warnings and Precautions (5.1)]*. Each of the following premedications should be given on the day of ONPATTRO infusion at least 60 minutes prior to the start of infusion:

- Intravenous corticosteroid (e.g., dexamethasone 10 mg, or equivalent)
- Oral acetaminophen (500 mg)
- Intravenous H1 blocker (e.g., diphenhydramine 50 mg, or equivalent)
- Intravenous H2 blocker (e.g., ranitidine 50 mg, or equivalent)

For premedications not available or not tolerated intravenously, equivalents may be administered orally.

For patients who are tolerating their ONPATTRO infusions but experiencing adverse reactions related to the corticosteroid premedication, the corticosteroid may be reduced by 2.5 mg increments to a minimum dose of 5 mg of dexamethasone (intravenous), or equivalent.

Some patients may require additional or higher doses of one or more of the premedications to reduce the risk of IRRs *[see Warnings and Precautions (5.1)]*.

2.3 Preparation Instructions

ONPATTRO must be filtered and diluted prior to intravenous infusion. The diluted solution for infusion should be prepared by a healthcare professional using aseptic technique as follows:

- Remove ONPATTRO from the refrigerator and allow to warm to room temperature. Do not shake or vortex.
- Inspect visually for particulate matter and discoloration. Do not use if discoloration or foreign particles are present. ONPATTRO is a white to off-white, opalescent, homogeneous solution. A white to off-white coating may be observed on the inner surface of the vial, typically at the liquid-headspace interface. Product quality is not impacted by presence of the white to off-white coating.
- Calculate the required dose of ONPATTRO based on the recommended weight-based dosage *[see Dosage and Administration (2.1)]*.
- Withdraw the entire contents of one or more vials into a single sterile syringe.
- Filter ONPATTRO through a sterile 0.45 micron polyethersulfone (PES) syringe filter into a sterile container.
- Withdraw the required volume of filtered ONPATTRO from the sterile container using a sterile syringe.
- Dilute the required volume of filtered ONPATTRO into an infusion bag containing 0.9% Sodium Chloride Injection, USP for a total volume of 200 mL. Use infusion bags that are di(2-ethylhexyl)phthalate-free (DEHP-free).
- Gently invert the bag to mix the solution. Do not shake. Do not mix or dilute with other drugs.
- Discard any unused portion of ONPATTRO.
- ONPATTRO does not contain preservatives. The diluted solution should be administered immediately after preparation. If not used immediately, store in the infusion bag at room temperature (up to 30°C [86°F]) for up to 16 hours (including infusion time). Do not freeze.

2.4 Infusion Instructions

- Use a dedicated line with an infusion set containing a 1.2 micron polyethersulfone (PES) in-line infusion filter. Use infusion sets and lines that are DEHP-free.
- Infuse the diluted solution of ONPATTRO intravenously, via an ambulatory infusion pump, over approximately 80 minutes, at an initial infusion rate of approximately 1 mL/min for the first 15 minutes, then increase to approximately 3 mL/min for the remainder of the infusion. The duration of infusion may be extended in the event of an IRR *[see Warnings and Precautions (5.1)]*.
- Administer only through a free-flowing venous access line. Monitor the infusion site for possible infiltration during drug administration. Suspected extravasation should be managed according to local standard practice for non-vesicants.
- Observe the patient during the infusion and, if clinically indicated, following the infusion *[see Warnings and Precautions (5.1)]*.
- After completion of the infusion, flush the intravenous administration set with 0.9% Sodium Chloride Injection, USP to ensure that all ONPATTRO has been administered.

3 DOSAGE FORMS AND STRENGTHS

Lipid Complex Injection: 10 mg/5 mL (2 mg/mL) white to off-white, opalescent, homogeneous solution in a single-dose vial.

4 CONTRAINDICATIONS

None.

5 WARNINGS AND PRECAUTIONS

5.1 Infusion-Related Reactions

Infusion-related reactions (IRRs) have been observed in patients treated with ONPATTRO. In clinical studies, all patients received premedication with a corticosteroid, acetaminophen, and antihistamines (H1 and H2 blockers) to reduce the risk of IRRs. In a controlled clinical study, 19% of ONPATTRO-treated patients experienced IRRs, compared to 9% of placebo-treated patients. Among ONPATTRO-treated patients who experienced an IRR, 79% experienced the first IRR within the first 2 infusions. The frequency of IRRs decreased over time. IRRs led to infusion interruption in 5% of patients. IRRs resulted in permanent discontinuation of ONPATTRO in less than 1% of patients in clinical studies. Across clinical studies, the most common symptoms (reported in greater than 2% of patients) of IRRs with ONPATTRO were flushing, back pain, nausea, abdominal pain, dyspnea, and headache *[see Adverse Reactions (6.1)]*. Severe hypotension and syncope have been reported as symptoms of IRRs in the expanded access program and postmarketing setting.

Patients should receive premedications on the day of ONPATTRO infusion, at least 60 minutes prior to the start of infusion *[see Dosage and Administration (2.2)]*. Monitor patients during the infusion for signs and symptoms of IRRs. If an IRR occurs, consider slowing or interrupting the ONPATTRO infusion and instituting medical management (e.g., corticosteroids or other symptomatic treatment), as clinically indicated. If the infusion is interrupted, consider resuming at a slower infusion rate only if symptoms have resolved. In the case of a serious or life-threatening IRR, the infusion should be discontinued and not resumed.

Some patients who experience IRRs may benefit from a slower infusion rate or additional or higher doses of one or more of the premedications with subsequent infusions to reduce the risk of IRRs *[see Dosage and Administration (2.2)]*.

5.2 Reduced Serum Vitamin A Levels and Recommended Supplementation

ONPATTRO treatment leads to a decrease in serum vitamin A levels. Supplementation at the recommended daily allowance of vitamin A is advised for patients taking ONPATTRO. Higher doses than the recommended daily allowance of vitamin A should not be given to try to achieve normal serum vitamin A levels during treatment with ONPATTRO, as serum vitamin A levels do not reflect the total vitamin A in the body.

Patients should be referred to an ophthalmologist if they develop ocular symptoms suggestive of vitamin A deficiency (e.g., night blindness).

6 ADVERSE REACTIONS

The following clinically significant adverse reactions are described elsewhere in the labeling:

- Infusion-Related Reactions *[see Warnings and Precautions (5.1)]*

6.1 Clinical Trials Experience

Because clinical studies are conducted under widely varying conditions, adverse reaction rates observed in the clinical studies of ONPATTRO cannot be directly compared to rates in the clinical studies of another drug and may not reflect the rates observed in practice.

A total of 224 patients with polyneuropathy caused by hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) received ONPATTRO in the placebo-controlled and open-label clinical studies, including 186 patients exposed for at least 1 year, 137 patients exposed for at least 2 years, and 52 patients exposed for at least 3 years. In the placebo-controlled study, 148 patients received ONPATTRO for up to 18 months (mean exposure 17.7 months). Baseline demographic and disease characteristics were generally similar between treatment groups. The median age of study patients was 62 years and 74% were male. Seventy-two percent of study patients were Caucasian, 23% were Asian, 2% were Black, and 2% were reported as other. At baseline, 46% of patients were in Stage 1 of the disease and 53% were in Stage 2. Forty-three percent of patients had Val30Met mutations in the transthyretin gene; the remaining patients had 38 other point mutations. Sixty-two percent of ONPATTRO-treated patients had non-Val30Met mutations, compared to 48% of the placebo-treated patients.

Upper respiratory tract infections and infusion-related reactions were the most common adverse reactions. One patient (0.7%) discontinued ONPATTRO because of an infusion-related reaction.

Patients were instructed to take the recommended daily allowance of vitamin A *[see Warnings and Precautions (5.2)]*. Sixty-four percent of patients treated with ONPATTRO had normal vitamin A levels at baseline, and 99% of those with a normal baseline developed low vitamin A levels. In one case, the decreased vitamin A level was reported as an adverse reaction.

Table 1 lists the adverse reactions that occurred in at least 5% of patients in the ONPATTRO-treated group and that occurred at least 3% more frequently than in the placebo-treated group in the randomized controlled clinical trial.

Table 1: Adverse Reactions from the Placebo-Controlled Trial that Occurred in at Least 5% of ONPATTRO-treated Patients and at Least 3% More Frequently than in Placebo-treated Patients

Adverse Reaction	ONPATTRO N=148 %	Placebo N=77 %
Upper respiratory tract infections [a]	29	21
Infusion-related reaction [b]	19	9
Dyspepsia	8	4
Dyspnea [c, d]	8	0
Muscle spasms [c]	8	1
Arthralgia [c]	7	0
Erythema [c]	7	3
Bronchitis [e]	7	3
Vertigo	5	1

[a] Includes nasopharyngitis, upper respiratory tract infection, respiratory tract infection, pharyngitis, rhinitis, sinusitis, viral upper respiratory tract infection, upper respiratory tract congestion.
[b] Infusion-related reaction symptoms include, but are not limited to: arthralgia or pain (including back, neck, or musculoskeletal pain), flushing (including erythema of face or skin warm), nausea, abdominal pain, dyspnea or cough,

chest discomfort or chest pain, headache, rash, chills, dizziness, fatigue, increased heart rate or palpitations, hypotension, hypertension, facial edema.

[c] Not part of an infusion-related reaction.

[d] Includes dyspnea and exertional dyspnea.

[e] Includes bronchitis, bronchiolitis, bronchitis viral, lower respiratory tract infection, lung infection.

Four serious adverse reactions of atrioventricular (AV) heart block (2.7%) occurred in ONPATTRO-treated patients, including 3 cases of complete AV block. No serious adverse reactions of AV block were reported in placebo-treated patients.

Ocular adverse reactions that occurred in 5% or less of ONPATTRO-treated patients in the controlled clinical trial, but in at least 2% of ONPATTRO-treated patients, and more frequently than on placebo, include dry eye (5% vs. 3%), blurred vision (3% vs. 1%), and vitreous floaters (2% vs. 1%).

Extravasation was observed in less than 0.5% of infusions in clinical studies, including cases that were reported as serious. Signs and symptoms included phlebitis or thrombophlebitis, infusion or injection site swelling, dermatitis (subcutaneous inflammation), cellulitis, erythema or injection site redness, burning sensation, or injection site pain.

6.2 Immunogenicity

The detection of antibody formation is highly dependent on the sensitivity and specificity of the assay. In addition, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors, including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to ONPATTRO in the studies described below with the incidence of antibodies in other studies or to other products may be misleading.

Anti-drug antibodies to ONPATTRO were evaluated by measuring antibodies specific to PEG_{2000}-C-DMG, a lipid component exposed on the surface of ONPATTRO. In the placebo-controlled and open-label clinical studies, 7 of 194 (3.6%) patients with hATTR amyloidosis developed anti-drug antibodies during treatment with ONPATTRO. One additional patient had pre-existing anti-drug antibodies. There was no evidence of an effect of anti-drug antibodies on clinical efficacy, safety, or the pharmacokinetic or pharmacodynamic profiles of ONPATTRO. Although these data do not demonstrate an impact of anti-drug antibody development on the efficacy or safety of ONPATTRO in these patients, the available data are too limited to make definitive conclusions.

6.3 Postmarketing Experience

The following adverse reactions have been identified during postapproval use of ONPATTRO. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

Symptoms of infusion-related reactions have included syncope *[see Warnings and Precautions (5.1)]* and pruritus.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to ONPATTRO during pregnancy. Physicians are encouraged to enroll pregnant patients, or pregnant women may register themselves in the program by calling 1-877-256-9526 or by contacting alnylampregnancyprogram@iqvia.com.

Risk Summary

There are no available data on ONPATTRO use in pregnant women to inform a drug-associated risk of adverse developmental outcomes. ONPATTRO treatment leads to a decrease in serum vitamin A levels, and vitamin A supplementation is advised for patients taking ONPATTRO. Vitamin A is essential for normal embryofetal development; however, excessive levels of vitamin A are associated with adverse developmental effects. The effects on the fetus of a reduction in maternal serum TTR caused by ONPATTRO and of vitamin A supplementation are unknown *[see Clinical Pharmacology (12.2), Warnings and Precautions (5.2)]*.

In animal studies, intravenous administration of patisiran lipid complex (patisiran-LC) to pregnant rabbits resulted in developmental toxicity (embryofetal mortality and reduced fetal body weight) at doses that were also associated with maternal toxicity. No adverse developmental effects were observed when patisiran-LC or a rodent-specific (pharmacologically active) surrogate were administered to pregnant rats (see *Data*).

In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2 to 4% and 15 to 20%, respectively. The background risk of major birth defects and miscarriage for the indicated population is unknown.

Data

Animal Data

Intravenous administration of patisiran LC (0, 0.15, 0.50, or 1.5 mg/kg) or a rodent-specific (pharmacologically active) surrogate (1.5 mg/kg) to female rats every week for two weeks prior to mating and continuing throughout organogenesis resulted in no adverse effects on fertility or embryofetal development.

Intravenous administration of patisiran-LC (0, 0.1, 0.3, or 0.6 mg/kg) to pregnant rabbits every week during the period of organogenesis produced no adverse effects on embryofetal development. In a separate study, patisiran-LC (0, 0.3, 1, or 2 mg/kg), administered to pregnant rabbits every week during the period of organogenesis, resulted in embryofetal mortality and reduced fetal body weight at the mid and high doses, which were associated with maternal toxicity.

Intravenous administration of patisiran-LC (0, 0.15, 0.50, or 1.5 mg/kg) or a rodent-specific surrogate (1.5 mg/kg) to pregnant rats every week throughout pregnancy and lactation resulted in no adverse developmental effects on the offspring.

8.2 Lactation

Risk Summary

There is no information regarding the presence of ONPATTRO in human milk, the effects on the breastfed infant, or the effects on milk production. The developmental and health benefits of breastfeeding should be considered along with the

mother's clinical need for ONPATTRO and any potential adverse effects on the breastfed infant from ONPATTRO or from the underlying maternal condition.

In lactating rats, patisiran was not detected in milk; however, the lipid components (DLin-MC3-DMA and PEG_{2000}-C-DMG) were present in milk.

8.4 Pediatric Use

Safety and effectiveness in pediatric patients have not been established.

8.5 Geriatric Use

No dose adjustment is required in patients ≥65 years old *[see Clinical Pharmacology (12.3)]*. A total of 62 patients ≥65 years of age, including 9 patients ≥75 years of age, received ONPATTRO in the placebo-controlled study. No overall differences in safety or effectiveness were observed between these patients and younger patients, but greater sensitivity of some older individuals cannot be ruled out.

8.6 Hepatic Impairment

No dose adjustment is necessary in patients with mild hepatic impairment (bilirubin ≤1 x ULN and AST >1 x ULN, or bilirubin >1.0 to 1.5 x ULN) *[see Clinical Pharmacology (12.3)]*. ONPATTRO has not been studied in patients with moderate or severe hepatic impairment.

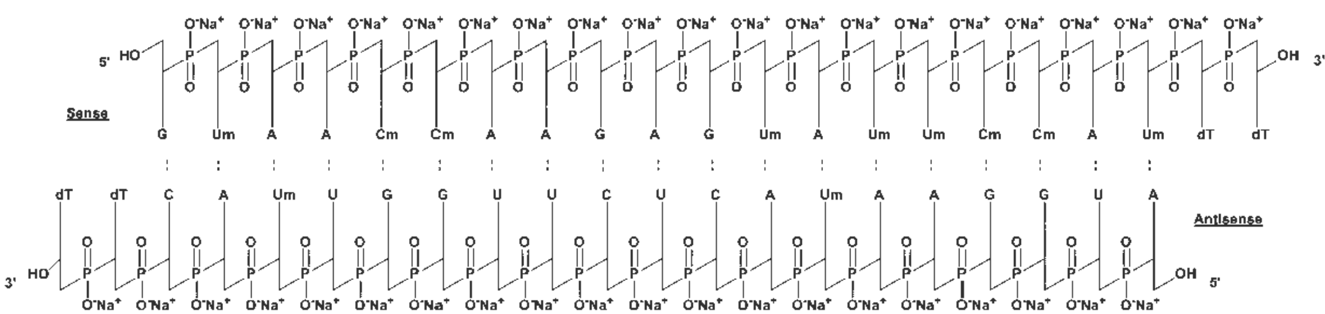
8.7 Renal Impairment

No dose adjustment is necessary in patients with mild or moderate renal impairment (estimated glomerular filtration rate [eGFR] ≥30 to <90 mL/min/1.73m^2) *[see Clinical Pharmacology (12.3)]*. ONPATTRO has not been studied in patients with severe renal impairment or end-stage renal disease.

11 DESCRIPTION

ONPATTRO contains patisiran, a double-stranded small interfering ribonucleic acid (siRNA), formulated as a lipid complex for delivery to hepatocytes. Patisiran specifically binds to a genetically conserved sequence in the 3' untranslated region (3'UTR) of mutant and wild-type transthyretin (TTR) messenger RNA (mRNA).

The structural formula is:



A, adenosine; C, cytidine; G, guanosine; U, uridine; Cm, 2'-*O*-methylcytidine; Um, 2'-*O*-methyluridine; dT, thymidine

ONPATTRO is supplied as a sterile, preservative-free, white to off-white, opalescent, homogeneous solution for intravenous infusion in a single-dose glass vial. Each 1 mL of solution contains 2 mg of patisiran (equivalent to 2.1 mg of patisiran sodium). Each 1 mL also contains 6.2 mg cholesterol USP, 13.0 mg (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-

tetraen-19-yl-4-(dimethylamino) butanoate (DLin-MC3-DMA), 3.3 mg 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1.6 mg α-(3'-{[1,2-di(myristyloxy)propanoxy] carbonylamino}propyl)-ω-methoxy, polyoxyethylene (PEG_{2000}-C-DMG), 0.2 mg potassium phosphate monobasic anhydrous NF, 8.8 mg sodium chloride USP, 2.3 mg sodium phosphate dibasic heptahydrate USP, and Water for Injection USP. The pH is ~7.0.

The molecular formula of patisiran sodium is $C_{412}\ H_{480}\ N_{148}\ Na_{40}\ O_{290}\ P_{40}$ and the molecular weight is 14304 Da.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Patisiran is a double-stranded siRNA that causes degradation of mutant and wild-type TTR mRNA through RNA interference, which results in a reduction of serum TTR protein and TTR protein deposits in tissues.

12.2 Pharmacodynamics

The pharmacodynamic effects of ONPATTRO were evaluated in hATTR amyloidosis patients treated with 0.3 mg/kg ONPATTRO via intravenous infusion once every 3 weeks.

Mean serum TTR was reduced by approximately 80% within 10 to 14 days after a single dose. With repeat dosing every 3 weeks, mean reductions of serum TTR after 9 and 18 months of treatment were 83% and 84%, respectively. The mean maximum reduction of serum TTR over 18 months was 88%. Similar TTR reductions were observed regardless of TTR mutation, sex, age, race, or prior liver transplantation. In a dose-ranging study, greater TTR reduction was maintained over the dosing interval with the recommended dosing regimen of 0.3 mg/kg every 3 weeks compared to 0.3 mg/kg every 4 weeks.

Serum TTR is a carrier of retinol binding protein, which is involved in the transport of vitamin A in the blood. Mean reductions in serum retinol binding protein of 45% and serum vitamin A of 62% were observed over 18 months *[see Warnings and Precautions (5.2)]*.

12.3 Pharmacokinetics

Following a single intravenous administration, systemic exposure to patisiran increases in a linear and dose-proportional manner over the range of 0.01 to 0.5 mg/kg. Greater than 95% of patisiran in the circulation is associated with the lipid complex. At the recommended dosing regimen of 0.3 mg/kg every 3 weeks, steady state is reached by 24 weeks of treatment. The estimated mean ± SD steady state peak concentrations (C_{max}), trough concentrations (C_{trough}), and area under the curve (AUC_{τ}) were 7.15 ± 2.14 μg/mL, 0.021 ± 0.044 μg/mL, and 184 ± 159 μg·h/mL, respectively. The accumulation of AUC_{τ} was 3.2-fold at steady state, compared to the first dose. In the placebo-controlled study, inter-patient variability in patisiran exposure did not result in differences in clinical efficacy (mNIS+7 change from baseline) or safety (adverse events, serious adverse events).

<u>Distribution</u>

Plasma protein binding of ONPATTRO is low, with ≤2.1% binding observed *in vitro* with human serum albumin and human α1-acid glycoprotein. ONPATTRO distributes primarily to the liver. At the recommended dosing regimen of 0.3 mg/kg every 3 weeks, the mean ± SD steady state volume of distribution of patisiran (V_{ss}) was 0.26 ± 0.20 L/kg.

Elimination

The terminal elimination half-life (mean ± SD) of patisiran is 3.2 ± 1.8 days. Patisiran is mainly cleared through metabolism, and the total body clearance (mean ± SD) at steady state (CL_{ss}) is 3.0 ± 2.5 mL/h/kg.

Metabolism

Patisiran is metabolized by nucleases to nucleotides of various lengths.

Excretion

Less than 1% of the administered dose of patisiran is excreted unchanged into urine.

Specific Populations

Age, race (non-Caucasian vs. Caucasian), sex, and prior liver transplantation had no impact on the steady state pharmacokinetics of patisiran or TTR reduction. Population pharmacokinetic and pharmacodynamic analyses indicated no impact of mild or moderate renal impairment (eGFR ≥30 to <90 mL/min/1.73m^2) or mild hepatic impairment (bilirubin ≤1 x ULN and AST >1 x ULN, or bilirubin >1.0 to 1.5 x ULN) on patisiran exposure or TTR reduction. ONPATTRO has not been studied in patients with severe renal impairment, end-stage renal disease, or moderate or severe hepatic impairment.

Drug Interaction Studies

No formal clinical drug interaction studies have been performed. The components of ONPATTRO are not inhibitors or inducers of cytochrome P450 enzymes or transporters at clinically relevant plasma concentrations. Patisiran is not a substrate of cytochrome P450 enzymes. In a population pharmacokinetic analysis, concomitant use of strong or moderate CYP3A inducers and inhibitors did not impact the pharmacokinetic parameters of patisiran. ONPATTRO is not expected to cause drug-drug interactions or to be affected by inhibitors or inducers of cytochrome P450 enzymes.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Patisiran-LC was not carcinogenic in TgRasH2 mice when administered at intravenous (IV) doses of 0, 0.5, 2, or 6 mg/kg every two weeks for 26 weeks.

Mutagenesis

Patisiran-LC was negative for genotoxicity in *in vitro* (bacterial mutagenicity assay, chromosomal aberration assay in human peripheral blood lymphocytes) and *in vivo* (mouse bone marrow micronucleus) assays.

Impairment of Fertility

Intravenous (IV) administration of patisiran-LC (0, 0.03, 0.1, or 0.3 mg/kg) or a rodent-specific (pharmacologically active) surrogate (0.1 mg/kg) to male rats every two weeks prior to and throughout mating to untreated females produced no adverse effects on fertility.

Intravenous administration of patisiran-LC (0, 0.15, 0.50, or 1.5 mg/kg) or a rodent-specific (pharmacologically active) surrogate (1.5 mg/kg) to female rats every week for two weeks prior to mating and continuing throughout organogenesis resulted in no adverse effects on fertility or on embryofetal development.

Intravenous administration of patisiran-LC (0, 0.3, 1, or 2 mg/kg) to adult monkeys every three weeks for 39 weeks produced no adverse effects on male reproductive organs or on sperm morphology or count.

14 CLINICAL STUDIES

The efficacy of ONPATTRO was demonstrated in a randomized, double-blind, placebo-controlled, multicenter clinical trial in adult patients with polyneuropathy caused by hATTR amyloidosis (NCT 01960348). Patients were randomized in a 2:1 ratio to receive ONPATTRO 0.3 mg/kg (N=148) or placebo (N=77), respectively, via intravenous infusion once every 3 weeks for 18 months. All patients received premedication with a corticosteroid, acetaminophen, and H1 and H2 blockers. Ninety-three percent of ONPATTRO-treated patients and 62% of placebo-treated patients completed 18 months of the assigned treatment.

The primary efficacy endpoint was the change from baseline to Month 18 in the modified Neuropathy Impairment Score +7 (mNIS+7). The mNIS+7 is an objective assessment of neuropathy and comprises the NIS and Modified +7 (+7) composite scores. In the version of the mNIS+7 used in the trial, the NIS objectively measures deficits in cranial nerve function, muscle strength, and reflexes, and the +7 assesses postural blood pressure, quantitative sensory testing, and peripheral nerve electrophysiology. The maximum possible score was 304 points, with higher scores representing a greater severity of disease.

The clinical meaningfulness of effects on the mNIS+7 was assessed by the change from baseline to Month 18 in Norfolk Quality of Life-Diabetic Neuropathy (QoL-DN) total score. The Norfolk QoL-DN scale is a patient-reported assessment that evaluates the subjective experience of neuropathy in the following domains: physical functioning/large fiber neuropathy, activities of daily living, symptoms, small fiber neuropathy, and autonomic neuropathy. The version of the Norfolk QoL-DN that was used in the trial had a total score range from -4 to 136, with higher scores representing greater impairment.

The changes from baseline to Month 18 on both the mNIS+7 and the Norfolk QoL-DN significantly favored ONPATTRO (Table 2, Figure 1 and Figure 3). The distributions of changes in mNIS+7 and Norfolk QoL-DN scores from baseline to Month 18 by percent of patients are shown in Figure 2 and Figure 4, respectively.

The changes from baseline to Month 18 in modified body mass index (mBMI) and gait speed (10-meter walk test) significantly favored ONPATTRO (Table 2).

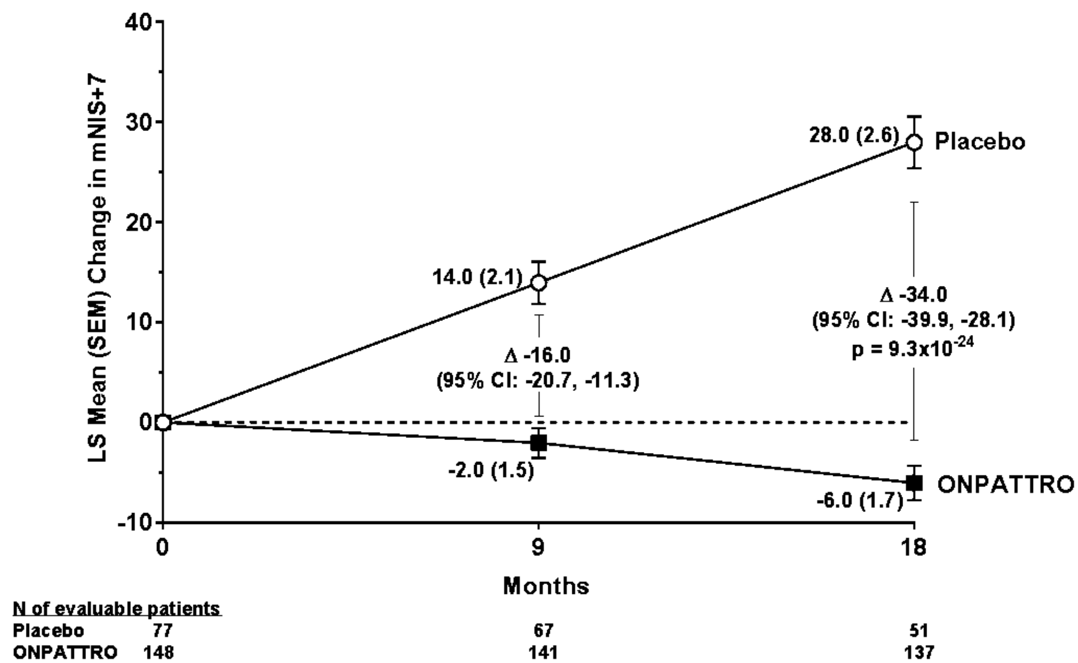
Table 2: Clinical Efficacy Results from the Placebo-Controlled Study

Endpoint[a]	Baseline, Mean (SD)		Change from Baseline to Month 18, LS Mean (SEM)		ONPATTRO-Placebo Treatment Difference, LS Mean (95% CI)	*p*-value
	ONPATTRO N=148	Placebo N=77	ONPATTRO	Placebo		
Primary						
mNIS+7 [b]	80.9 (41.5)	74.6 (37.0)	-6.0 (1.7)	28.0 (2.6)	-34.0 (-39.9, -28.1)	$p<0.001$
Secondary						

Endpoint[a]	Baseline, Mean (SD)		Change from Baseline to Month 18, LS Mean (SEM)		ONPATTRO-Placebo Treatment Difference, LS Mean (95% CI)	*p*-value
	ONPATTRO N=148	Placebo N=77	ONPATTRO	Placebo		
Norfolk QoL-DN [b]	59.6 (28.2)	55.5 (24.3)	-6.7 (1.8)	14.4 (2.7)	-21.1 (-27.2, -15.0)	p<0.001
10-meter walk test (m/sec) [c]	0.80 (0.40)	0.79 (0.32)	0.08 (0.02)	-0.24 (0.04)	0.31 (0.23, 0.39)	p<0.001
mBMI [d]	970 (210)	990 (214)	-3.7 (9.6)	-119 (14.5)	116 (82, 149)	p<0.001

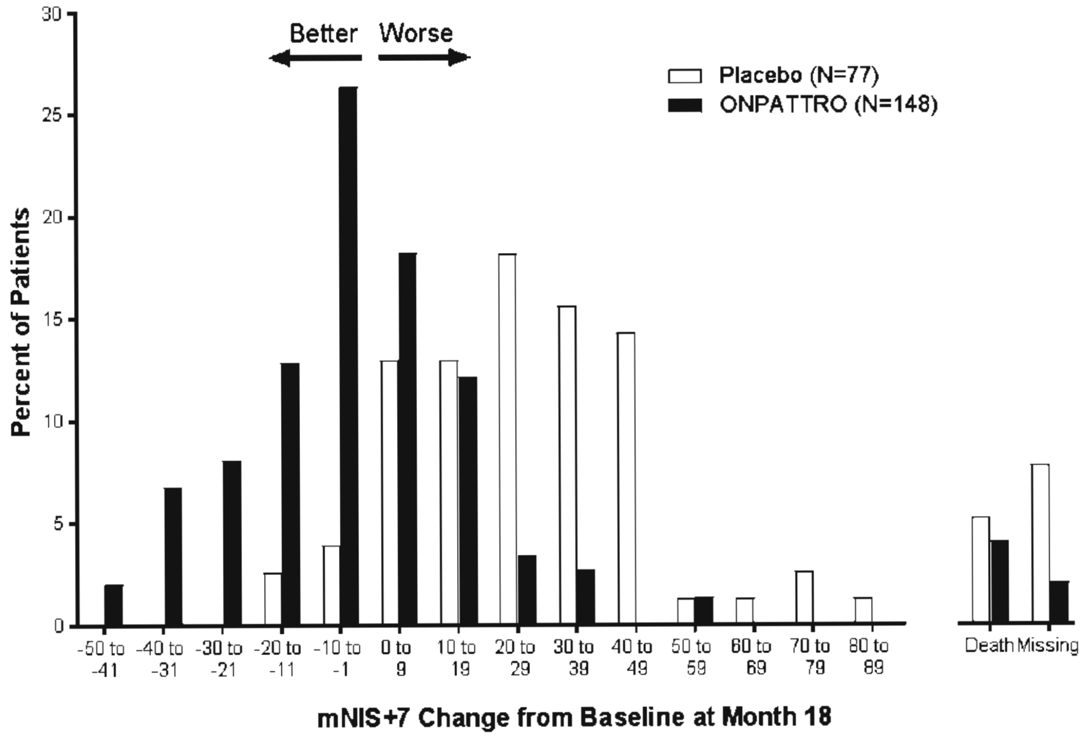
CI, confidence interval; LS, least squares; mBMI, modified body mass index; mNIS, modified Neuropathy Impairment Score; QoL-DN, Quality of Life – Diabetic Neuropathy; SD, standard deviation; SEM, standard error of the mean
[a] All endpoints analyzed using the mixed-effect model repeated measures (MMRM) method.
[b] A lower value indicates less impairment/fewer symptoms.
[c] A higher number indicates less disability/less impairment.
[d] mBMI: body mass index (BMI; kg/m^2) multiplied by serum albumin (g/L); a higher number indicates better nutritional status.

Figure 1: Change from Baseline in mNIS+7



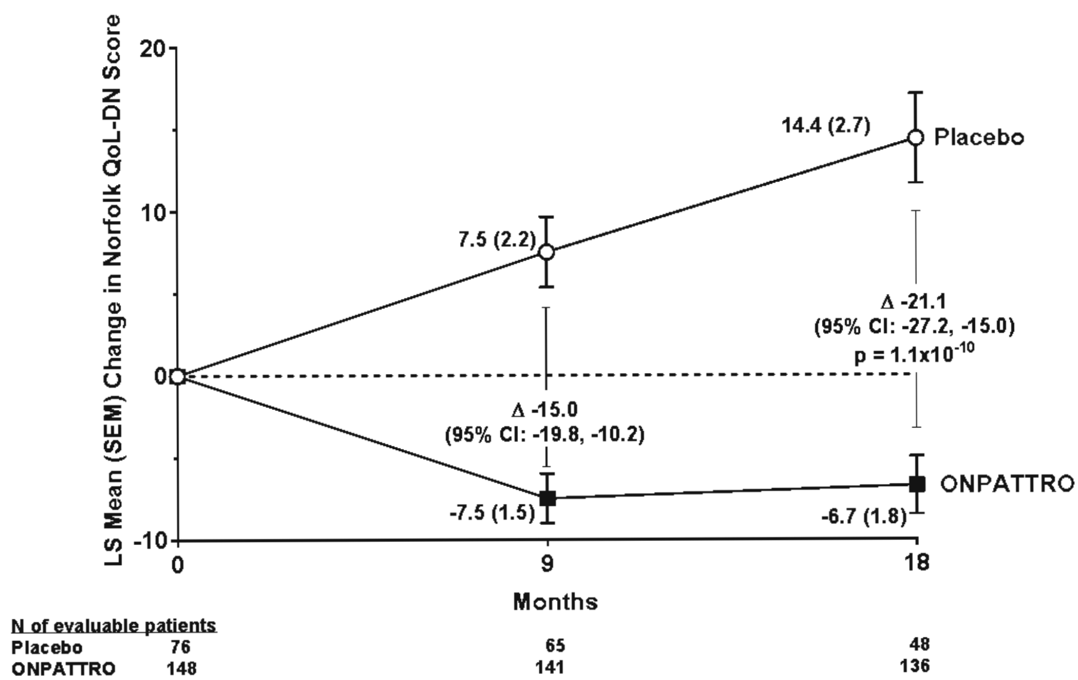
A decrease in mNIS+7 indicates improvement.
Δ indicates between-group treatment difference, shown as the LS mean difference (95% CI) for ONPATTRO – placebo.

Figure 2: Histogram of mNIS+7 Change from Baseline at Month 18



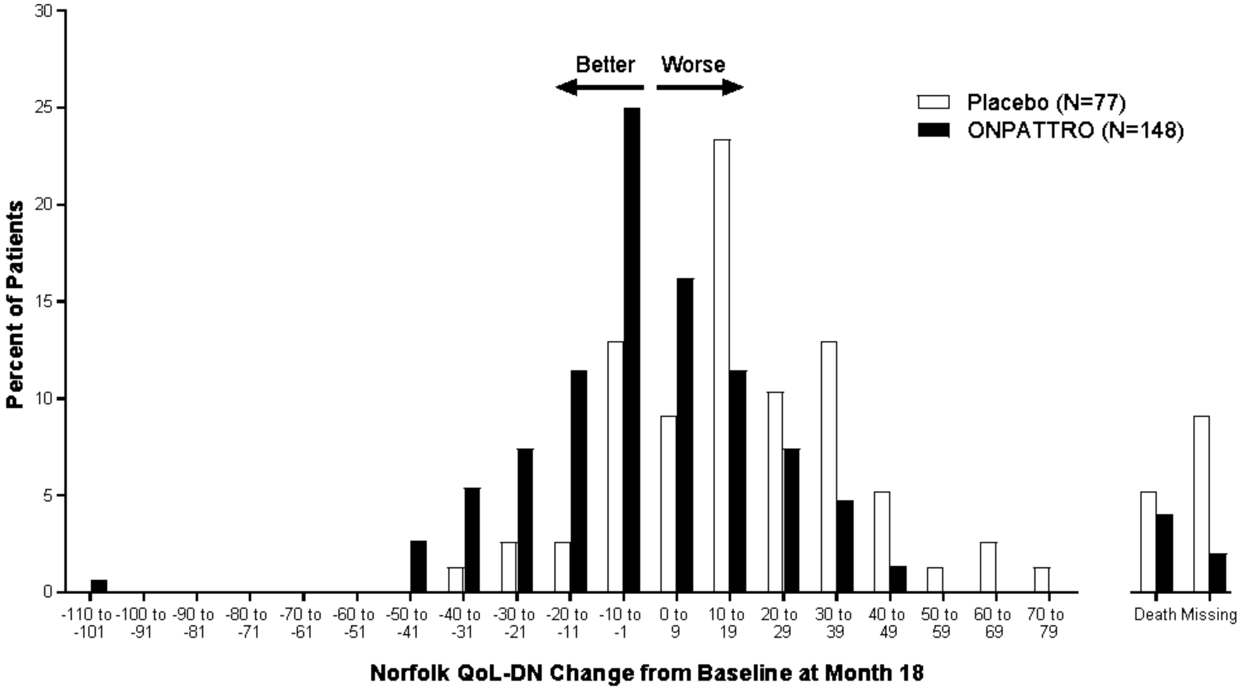
mNIS+7 change scores are rounded to the nearest whole number; last available post-baseline scores were used. Categories are mutually exclusive; patients who died before 18 months are summarized in the "Death" category only.

Figure 3: Change from Baseline in Norfolk QoL-DN Score



A decrease in Norfolk QoL-DN score indicates improvement.
Δ indicates between-group treatment difference, shown as the LS mean difference (95% CI) for ONPATTRO – placebo.

Figure 4: Histogram of Norfolk QoL-DN Change from Baseline at Month 18



Norfolk QoL-DN change scores are rounded to the nearest whole number; last available post-baseline scores were used.
Categories are mutually exclusive; patients who died before 18 months are summarized in the "Death" category only.

Patients receiving ONPATTRO experienced similar improvements relative to placebo in mNIS+7 and Norfolk QoL-DN score across all subgroups including age, sex, race, region, NIS score, Val30Met mutation status, and disease stage.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

ONPATTRO is a sterile, preservative-free, white to off-white, opalescent, homogeneous solution for intravenous infusion supplied as a 10 mg/5 mL (2 mg/mL) solution in a single-dose glass vial. The vial stopper is not made with natural rubber latex. ONPATTRO is available in cartons containing one single-dose vial each.

The NDC is: 71336-1000-1.

16.2 Storage and Handling

Store at 2°C to 8°C (36°F to 46°F). Do not freeze. Discard vial if it has been frozen.

If refrigeration is not available, ONPATTRO can be stored at room temperature up to 25°C (up to 77°F) for up to 14 days.

For storage conditions of ONPATTRO after dilution in the infusion bag, see Dosage and Administration (2.3).

17 PATIENT COUNSELING INFORMATION

Infusion-Related Reactions

Inform patients about the signs and symptoms of infusion-related reactions (e.g., flushing, dyspnea, chest pain, syncope, rash, increased heart rate, facial edema). Advise patients to contact their healthcare provider immediately if they experience signs and symptoms of infusion-related reactions *[see Warnings and Precautions (5.1)]*.

Recommended Vitamin A Supplementation

Inform patients that ONPATTRO treatment leads to a decrease in vitamin A levels measured in the serum. Instruct patients to take the recommended daily allowance of vitamin A. Advise patients to contact their healthcare provider if they experience ocular symptoms suggestive of vitamin A deficiency (e.g., night blindness) and refer them to an ophthalmologist if they develop these symptoms *[see Warnings and Precautions (5.2)]*.

Pregnancy

Instruct patients that if they are pregnant or plan to become pregnant while taking ONPATTRO they should inform their healthcare provider. Advise female patients of childbearing potential of the potential risk to the fetus. Encourage patients to enroll in the ONPATTRO pregnancy exposure registry if they become pregnant while taking ONPATTRO *[see Use in Specific Populations (8.1)]*.

Manufactured for: Alnylam Pharmaceuticals, Inc.
300 Third Street, Cambridge, MA 02142
By: Ajinomoto Althea, Inc.
11040 Roselle Street, San Diego, CA 92121

Exhibit C

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CERTIFICATE #2797.06

Test Report
Identification of Neutral Phospholipids in Onpattro

University of Texas M.D. Anderson Cancer Center

EAG Job #V1OSU897

Revision	Description	Date
0	Initial report.	March 14, 2024

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EXECUTIVE SUMMARY FOR
University of Texas M.D. Anderson Cancer Center

March 14, 2024

STUDY OBJECTIVE

V1OSU897 Qualitative screening of samples to determine the presence of neutral phospholipids.

SUMMARY OF ANALYTICAL RESULTS AND INTERPRETATIONS

Onpattro (Patisiran) Lipid Complex Injection (S2) was screened for neutral phospholipids by LC/MS. Excluding DSPC and lyso forms of DSPC (18:0 Lyso PC and 2-18:0 Lyso PC), the next two major neutral phospholipids found in S2 were identified as 18:0-20:0 PC and PSPC or 16:0-18:0 PC. The results are outlined below.

COMPOUND NAME	CAS NUMBER	MOLECULAR FORMULA	MASS (M/Z)	%AREA RELATIVE TO DSPC
1-stearoyl-2-arachidoyl-sn-glycerol-3-phosphocholine (18:0-20:0 PC)	61574-14-9	$C_{46}H_{92}NO_8P$	818.6633	7.14
1-palmitoyl-2-stearoyl-sn-glycerol-3-phosphocholine (PSPC, 16:0-18:0 PC)	59403-51-9	$C_{42}H_{84}NO_8P$	762.6010	2.87

SAMPLE LOG-IN

SAMPLE NUMBER	DESCRIPTION	DATE RECEIVED
S2	Onpattro (Patisiran) Lipid Complex Injection, GTIN: 00810559030003 S/N: 3768044361384, Lot: 02893276, Exp: NOV 2024, Qty: 10mg/5mL	05 Jan 2024

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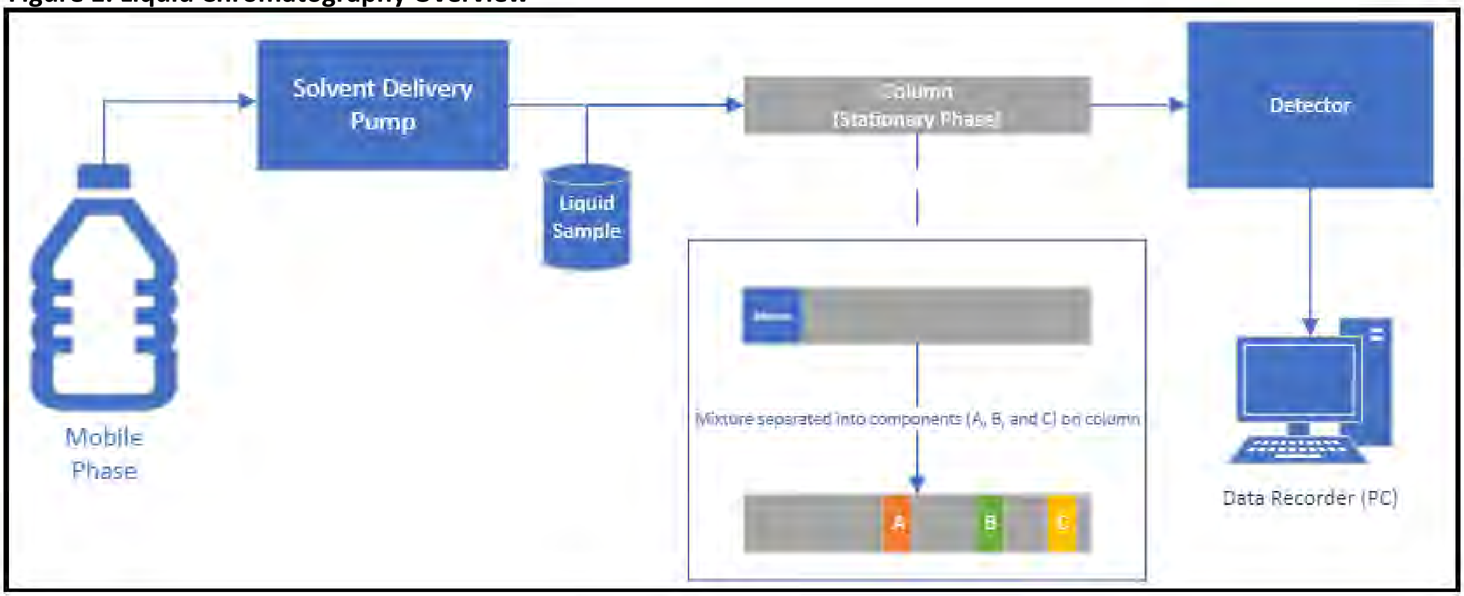
IDENTIFICATION OF NEUTRAL PHOSPHOLIPIDS IN ONPATTRO ANALYTICAL RESULTS AND INTERPRETATIONS

LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRY (LC/MS)

Liquid Chromatography/Mass Spectrometry (LC/MS) is an analytical chemistry technique used to characterize the structures of components in a complex matrix and quantify them. Liquid chromatography is used for the separation of a sample mixture into its components. A mixture of solvents or solutions, called the mobile phase, is forced at high pressure by a pump through a packed column, usually of coated silica particles, called the stationary phase. Components in the mixture are separated based on the difference in their affinities for the stationary phase and the mobile phase. The separated components can be detected and measured as they elute from the column. An overview of liquid chromatography showing the flow path of mobile phase and information is shown in Figure 1.

Figure 1. Liquid Chromatography Overview



In LC/MS, the detector is a mass spectrometer. The effluent from the chromatographic separation is sent to the ion source of a mass spectrometer where the resolved components are ionized and enter the mass spectrometer to be analyzed. During each scan of the mass spectrometer, the mass to charge ratios (m/z) of ions present are measured and plotted against their intensities. The sum of the intensities of all mass spectral peaks in each scan are plotted over time as they are separated and detected in what is called the Total Ion Chromatogram (TIC). Two other types of LC/MS chromatograms that can be generated from the same data as the TIC are Base Peak Chromatogram (BPC) and Extracted Ion Chromatogram (EIC). Base peak chromatograms plot only the most intense peak of each spectrum instead of the sum. Extracted ion chromatograms are reconstructed from the data focused on a single m/z of interest.

Coupled to a high-resolution mass spectrometer (HRMS) such as an orbitrap, LC/MS can be used to determine the molecular formula of components in a sample mixture from the measured m/z and isotopic distribution. In addition, tandem mass spectrometry or MS/MS can be utilized to characterize the structure of an ion. During MS/MS experiments, one or more isolation and fragmentation steps are applied allowing for structural information to be obtained based on the observed fragment ions. An instrument with more than one mass analyzer is necessary for an MS/MS experiment. For example, a Thermo Orbitrap ID-X, a tribrid mass spectrometer with three mass detectors/filters: quadrupole, ion trap, and orbitrap, can run MS/MS experiments. An instrument diagram for a Thermo Orbitrap ID-X is shown in Figure 2. MS/MS experiments are labeled as MS^n, where n is the number of mass filtration/fragmentation steps and MS^1 does not involve fragmentation. During full-scan MS experiments (MS^1), ions flow from the source through the quadrupole to the C-trap and are then injected into the orbitrap for mass analysis. During an MS/MS experiment, ions flow from the source through the quadrupole which is set to isolate a single precursor ion (typically 1.5 m/z isolation range). Following quadrupole mass filtration of the precursor ion, the C-trap allows the ions pass to the Ion-routing multipole (IRM), which functions as a collision cell. Ions are then subjected to higher-energy collisional dissociation (HCD) fragmentation by collision with an

inert gas such as Nitrogen. The resulting fragment ions are then be routed to the orbitrap for mass analysis in an MS^2 experiment. The instrument components utilized in an HCD MS^2 experiment are labeled in Figure 3. Collision-induced dissociation (CID) fragmentation, another method of collision-based fragmentation, takes place in the ion trap and employs Helium as the inert gas.

Figure 2. Intrument Diagram of a Thermo Orbitrap ID-X

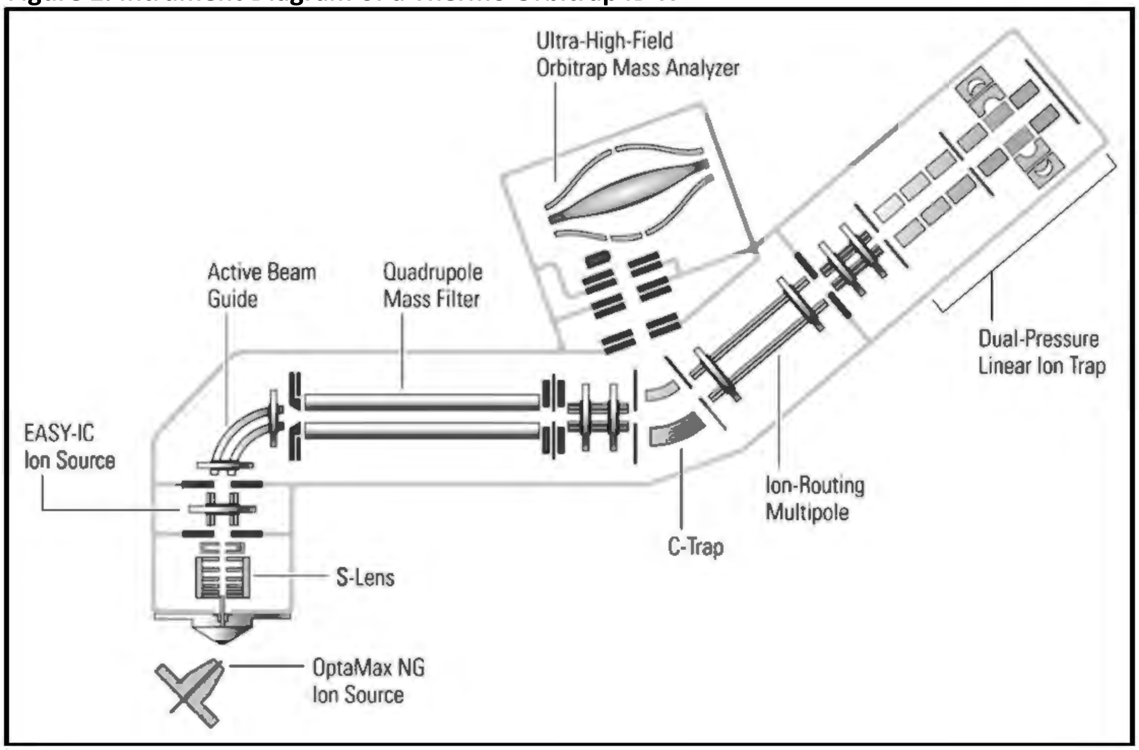
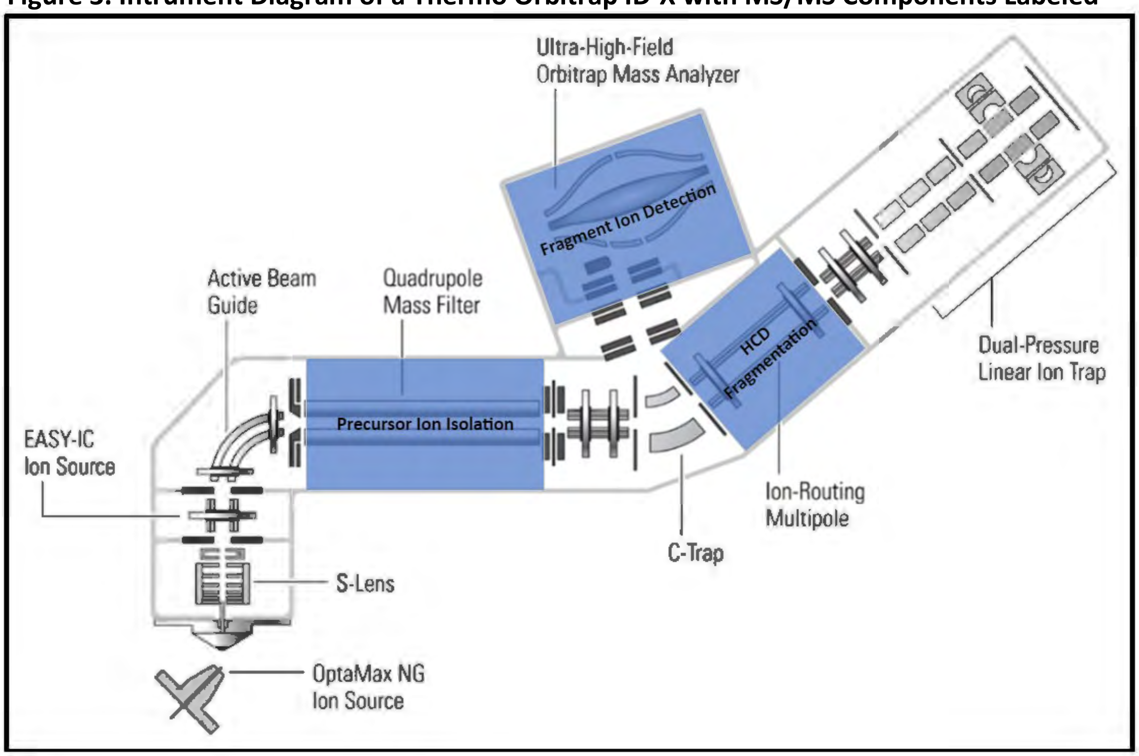


Figure 3. Intrument Diagram of a Thermo Orbitrap ID-X with MS/MS Components Labeled

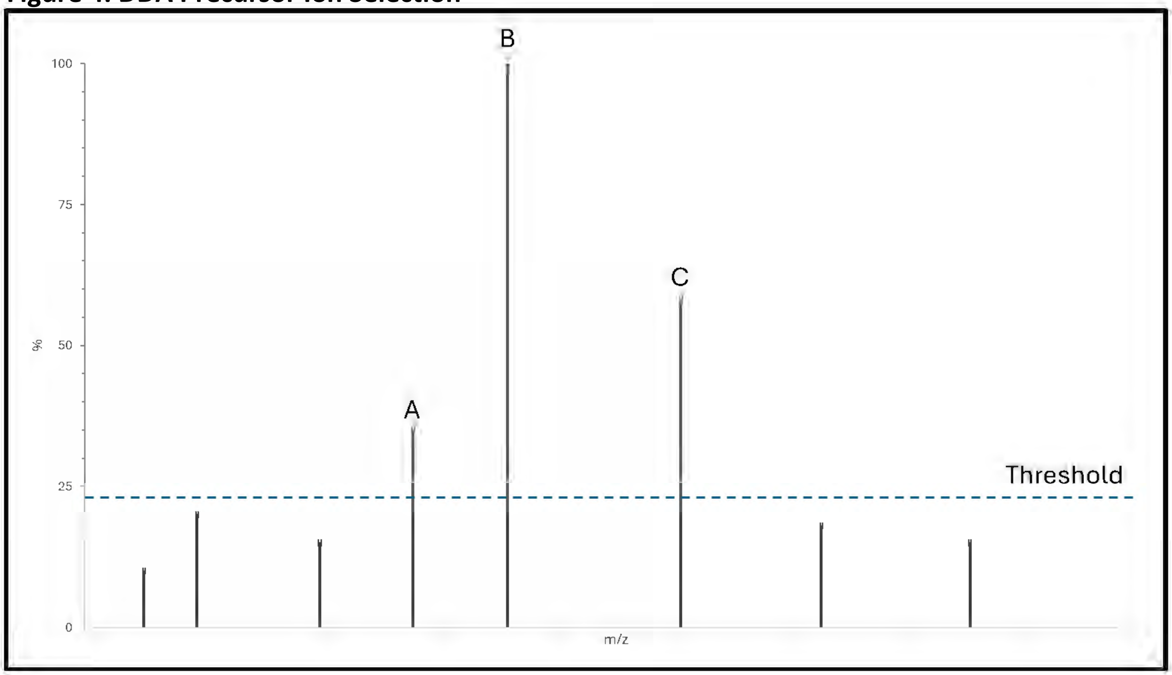


If an instrument has an ion trap, higher level MS/MS experiments can be conducted where fragments of fragments are detected. For example, in an MS^3 experiment, the precursor ion filtered by the quadrupole could be fragmented in the

IRM and sent to the ion trap; a specific m/z fragment ion can be trapped and sent back to the IRM for subsequent fragmentation followed by mass analysis by the orbitrap.

Data dependent acquisition (DDA) is an acquisition workflow where the MS instrument automatically switches from full-scan MS to MS/MS between scans based on the full-scan MS data.[1,2] When ions in the full-scan MS meet predefined criteria, such as exceeding an intensity threshold (Figure 4), the instrument selects those ions (e.g. A, B, and C) as target precursors for MS/MS scans. The instrument then performs MS/MS scans on the selected precursor ions in the time between full-scan MS during the duty cycle of the workflow. DDA workflows allow for untargeted acquisition of data to characterize and identify many components in a complex sample without prior knowledge of the sample components by the user.

Figure 4. DDA Precursor Ion Selection



Liquid Chromatography with Mass Spectrometry (LC/MS) Analysis of Onpattro

Onpattro (Patisiran) Lipid Complex Injection (S2, 10 mg/5 mL) was screened for neutral phospholipids by LC/MS. The as-received sample (S2) was diluted 1:1 in methanol (MeOH) and analyzed with a Thermo Orbitrap ID-X LC/MS instrument using a C30 column and mobile phases consisting of formic acid, ammonium formate, difluoroacetic acid (DFA), acetonitrile (ACN), water (H_2O), and isopropanol (IPA). Both full-scan MS^1 and DDA MS^2 data was collected with positive mode Electrospray Ionization (ESI). The instrument method parameters are outlined in Table 1.[3] The base peak chromatogram from the analysis of the sample is presented in Figure 5 below. 1,2-distearoyl-sn-glycero-3-phosphocholine (CAS 816-94-4), abbreviated as 18:0 PC or DSPC, was known to be present in S2. A standard of DSPC (Sigma Aldrich, PN: P1138) was analyzed alongside the sample for comparison using the same method. A stack of the EIC's (790.6324 m/z) for the sample and DSPC standard is shown in Figure 6. The HCD fragmentation of both the standard and sample gives a characteristic phosphocholine fragment ion of 184.0733 m/z as shown in the MS^2 of the standard in Figure 7. The structure of this PC fragment ion resulting from DSPC is given in Figure 8. This fragmentation pathway is known in the literature and can be applied to identify other neutral phospholipids containing the phosphocholine moiety.[4,5]

Table 1. LC/MS Method Parameters

INSTRUMENT	Thermo Orbitrap ID-X with Vanquish LC Stack	
COLUMN	Accucore C30, 150 x 2.1 mm, 2.6 µm	
COLUMN TEMPERATURE	50 °C	
AUTOSAMPLER TEMPERATURE	10 °C	
INJECTION VOLUME	2 µL	
SOLVENT SYSTEM	Mobile Phase A	10 mM Ammonium Formate, 0.1% DFA in 60:40 ACN:Water
	Mobile Phase B	10 mM Ammonium Formate, 0.1% DFA in 90:10 IPA:ACN
FLOW RATE	0.35 mL/min	
GRADIENT	Time (min)	% B
	0	30
	2	43
	2.1	55
	10	65
	13	85
	14	100
	16.5	100
	16.6	30
	22	30
MS DETECTION	Positive ESI 120-1300 m/z full-scan with DDA (HCD MS^2)	

Figure 5. LC/MS Base Peak Chromatogram of Sample S2

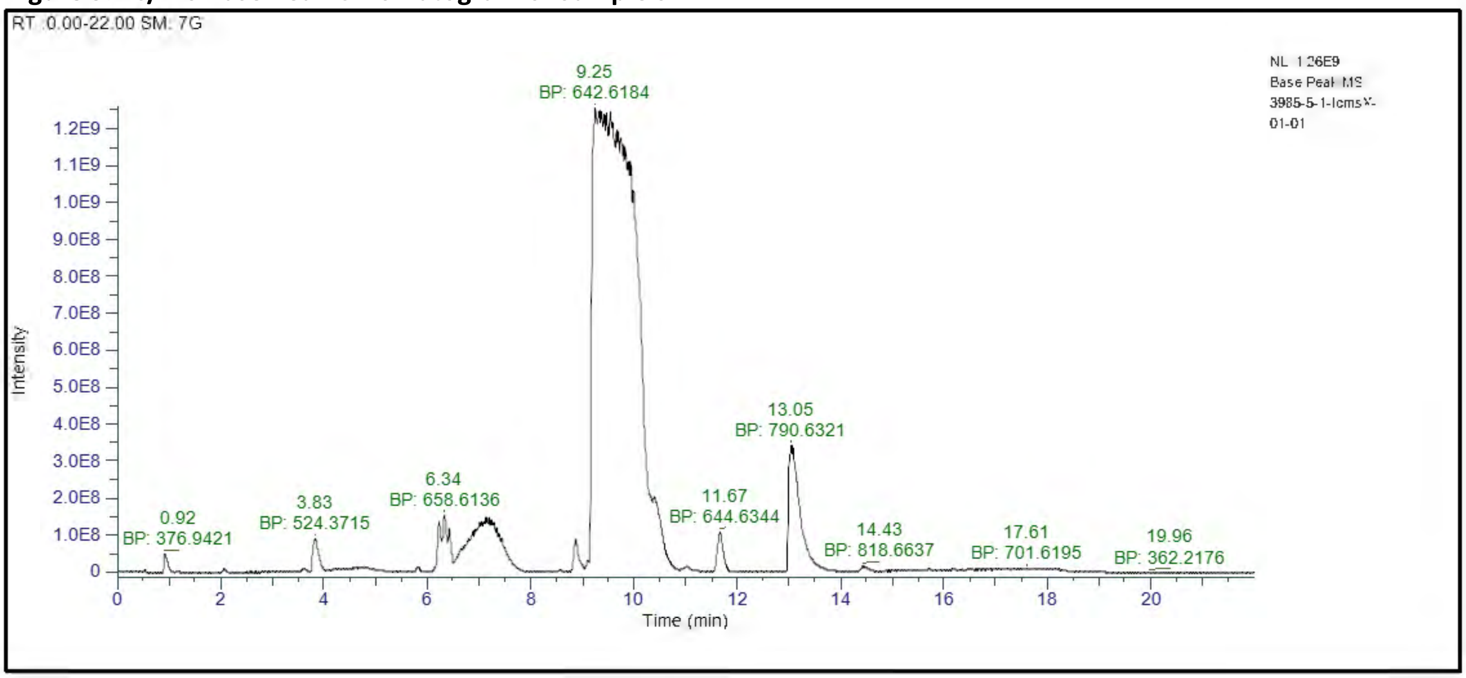


Figure 6. Extracted Ion Chromatogram Comparison of Sample S2 and DSPC Standard (790.6324 m/z)

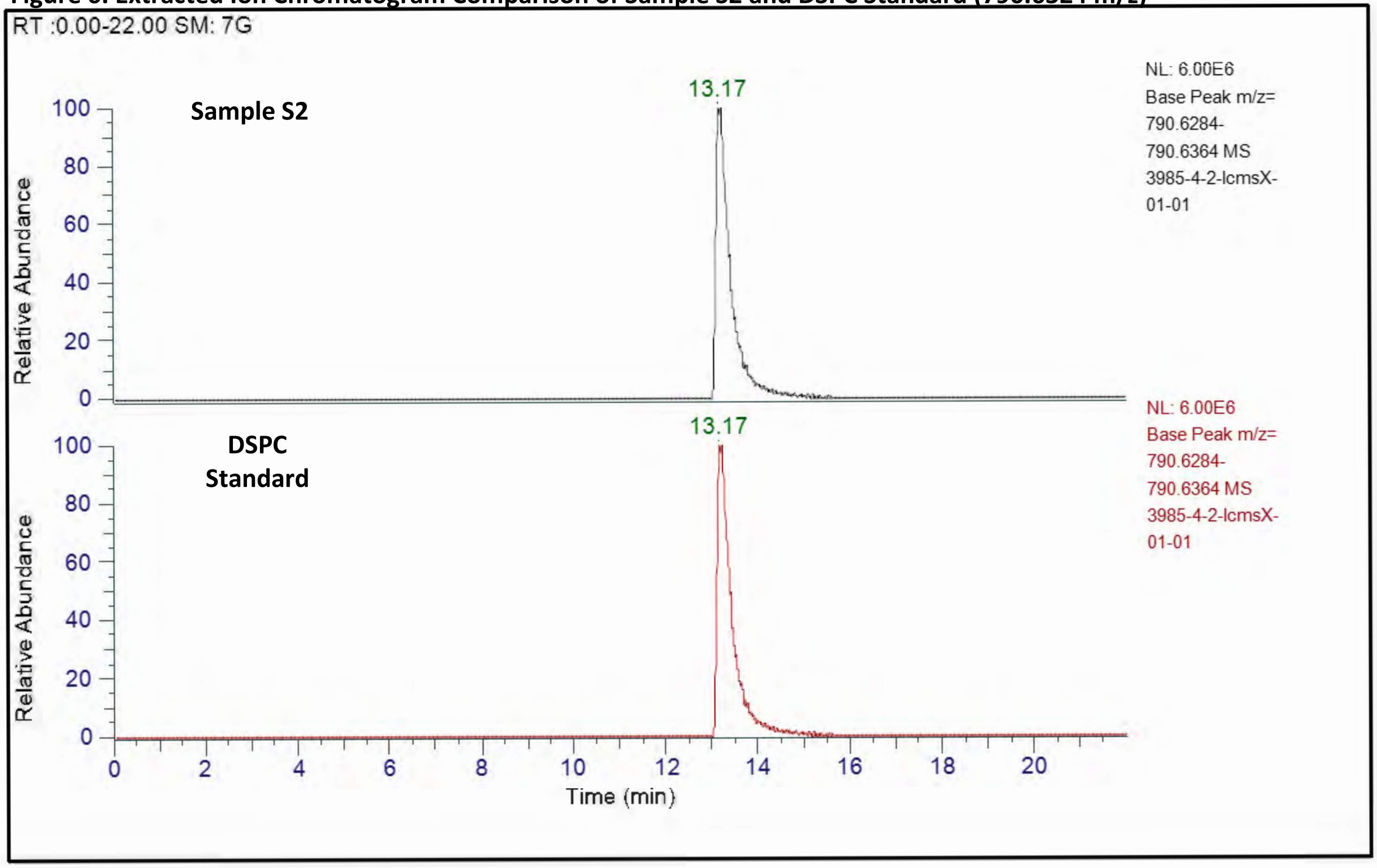


Figure 7. DSPC MS^2 Spectrum of 790.6326 m/z precursor ion

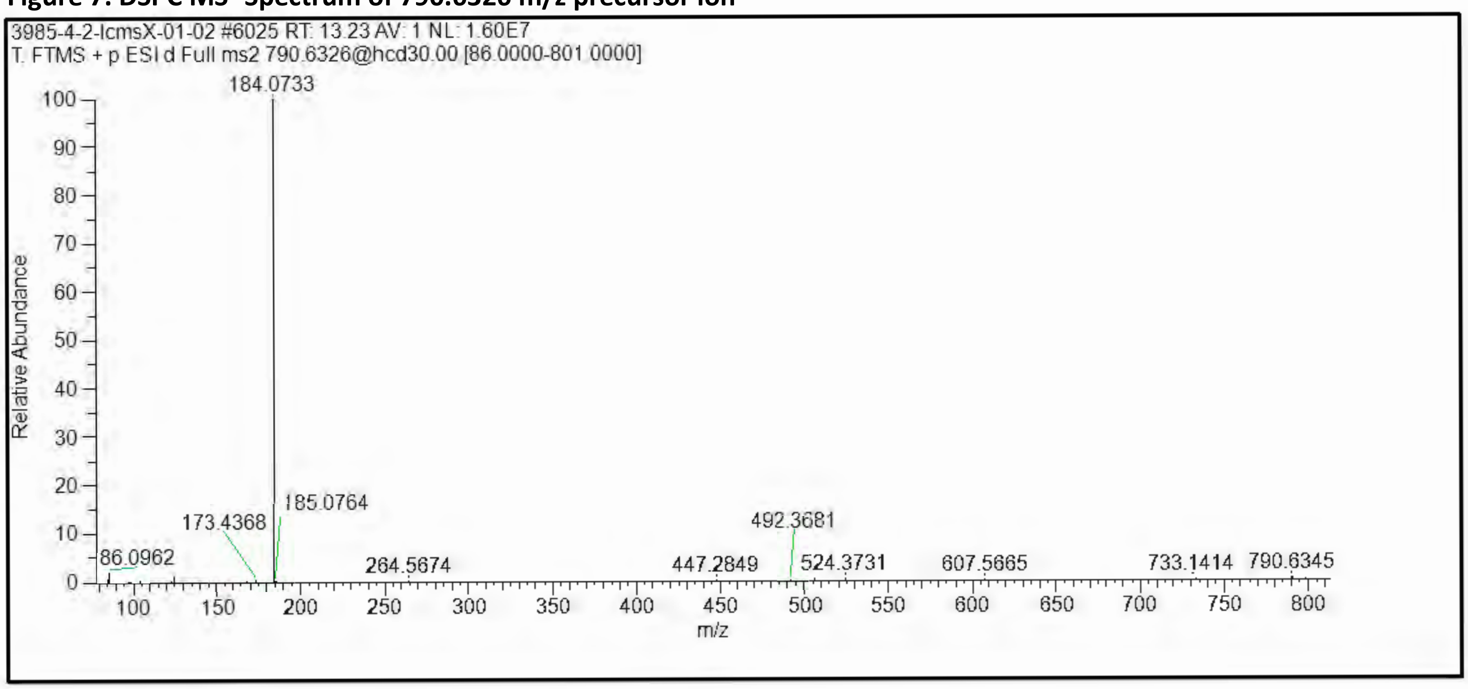
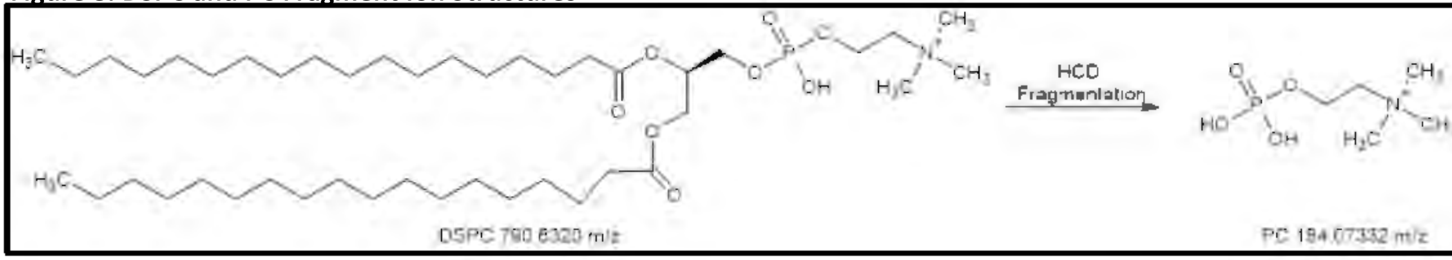


Figure 8. DSPC and PC Fragment Ion Structures



The acquired sample data was screened for neutral phospholipids using Compound Discoverer software. Based on these criteria, the results of this screen and the tentative neutral phospholipid assignments are outlined in Table 2. The EIC peak areas were compared to DSPC, and the relative abundances were approximated. Excluding DSPC and 18:0 Lyso PC (524.3711 m/z), the next two major neutral phospholipids found in S2 were identified as 18:0-20:0 PC and 16:0-18:0 PC (818.6633 m/z and 762.6010 m/z). An overlay of the 762.6010, 790.6317, and 818.6633 m/z EIC's for the major neutral phospholipids of interest is shown in Figure 9. These structures were assigned based on the formulas resulting from the observed ions for the $[M+H]^+$ adduct and the characteristic PC loss in the MS^2. The EIC and spectra for 16:0-18:0 PC in S2 are shown in Figure 10 and Figure 11. The EIC and spectra for 18:0-20:0 PC in S2 are shown in Figure 12 and Figure 13.

Table 2. Tentative List of Neutral Phospholipids Detected in Sample S2

Tentative Identification	CAS Number	Formula	m/z	Retention Time (min)	Area	%Area Relative to DSPC
1,2-distearoyl-sn-glycerol-3-phosphocholine (DSPC, 18:0 PC)	816-94-4	$C_{44}H_{88}NO_8P$	790.6317	13.04	8,863,876,149	-
1-stearoyl-2-hydroxy-sn-glycero-3-phosphocholine (18:0 Lyso PC)	19420-57-6	$C_{26}H_{54}NO_7P$	524.3711	3.82	984,238,457	11.10
1-stearoyl-2-arachidoyl-sn-glycerol-3-phosphocholine (18:0-20:0 PC)	61574-10-5	$C_{46}H_{92}NO_8P$	818.6633	14.44	632,438,590	7.14
1-palmitoyl-2-stearoyl-sn-glycerol-3-phosphocholine (PSPC, 16:0-18:0 PC)	59403-51-9	$C_{42}H_{84}NO_8P$	762.6010	11.85	254,789,333	2.87
2-stearoyl-sn-glycero-3-phosphocholine (2-18:0 Lyso PC)	4421-58-3	$C_{26}H_{54}NO_7P$	524.3713	3.61	109,916,240	1.24
1-heptadecanoyl-2-stearoyl-sn-glycero-3-phosphocholine (17:0-18:0 PC)	360550-42-1	$C_{43}H_{86}NO_8P$	776.6166	12.88	22,838,063	0.26
1-arachidoyl-2-hydroxy-sn-glycero-3-phosphocholine (20:0 Lyso PC)	108341-80-6	$C_{28}H_{58}NO_7P$	552.4028	4.80	9,433,454	0.11
1-hydroxy-2-palmitoyl-sn-glycero-3-phosphocholine (2-16:0 Lyso PC)	66757-27-5	$C_{24}H_{50}NO_7P$	496.3398	2.72	5,757,758	0.06

Figure 9. Extracted Ion Chromatogram Overlay of Major Neutral Phospholipids in Sample S2

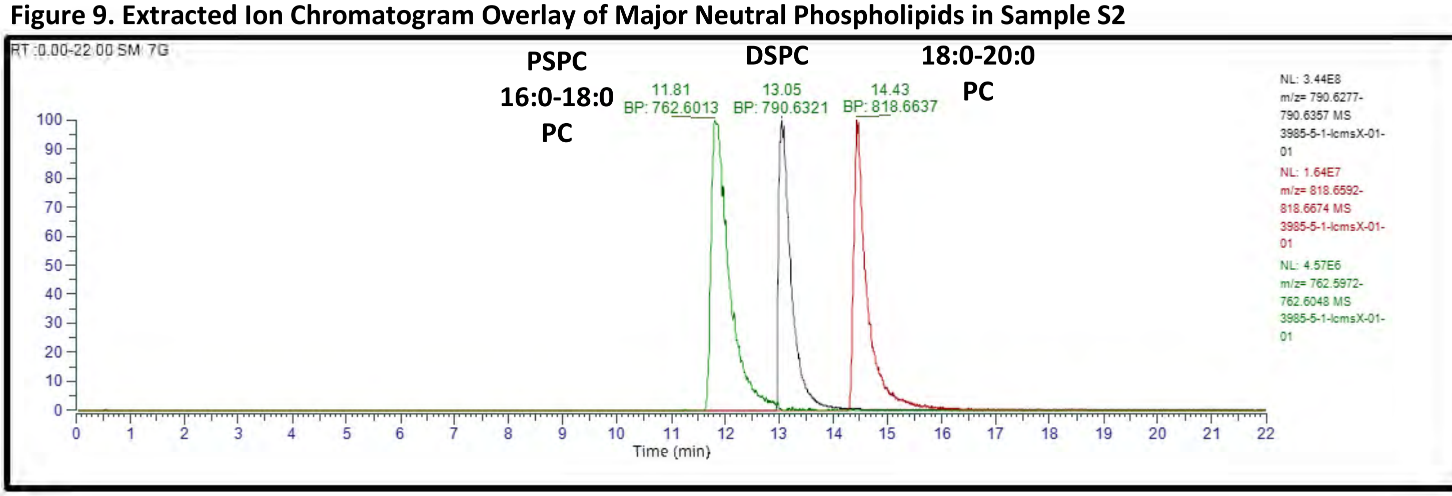


Figure 10. Extracted Ion Chromatogram and Corresponding Mass Spectrum for 762.6012 m/z

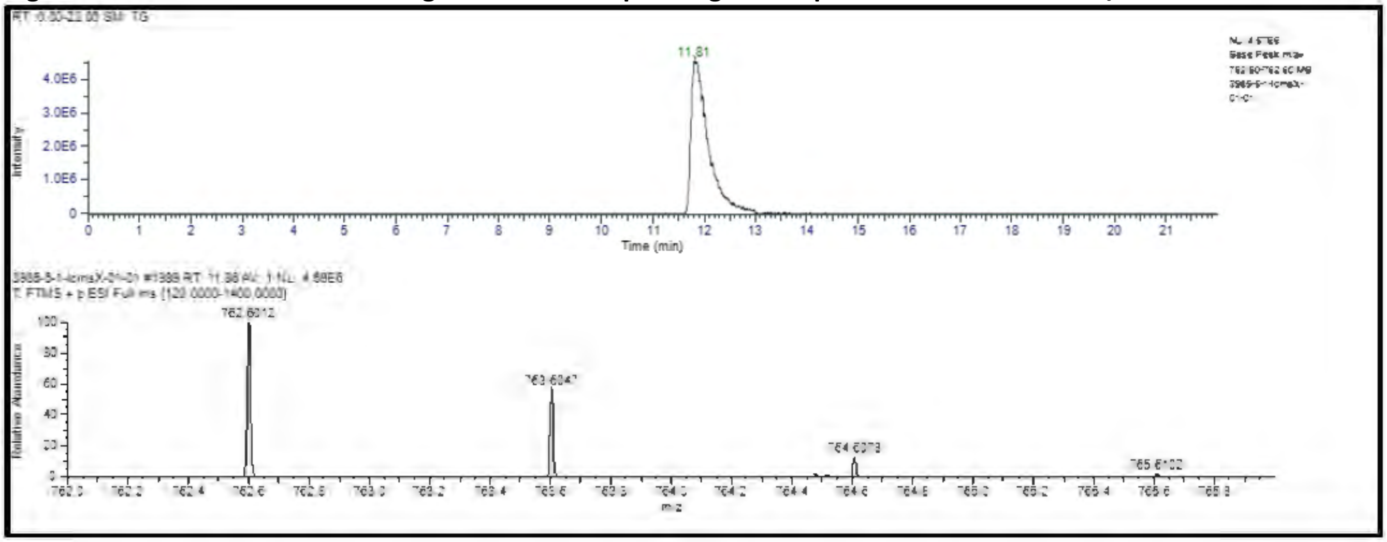


Figure 11. Fragmentation Mass Spectrum (MS^2) of 762.6012 m/z precursor ion

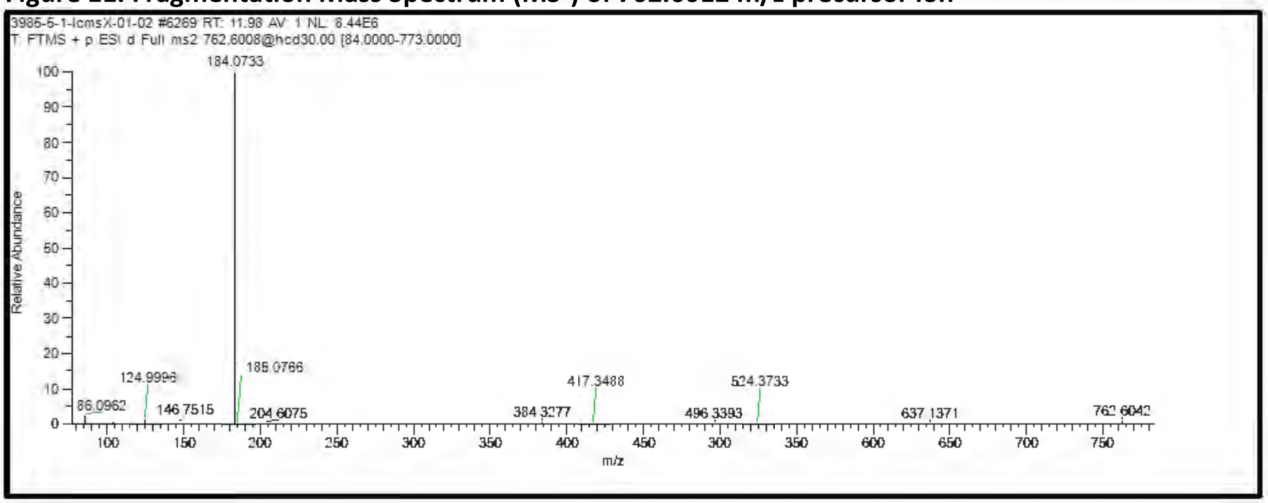


Figure 12. Extracted Ion Chromatogram and Corresponding Mass Spectrum for 818.6633 m/z

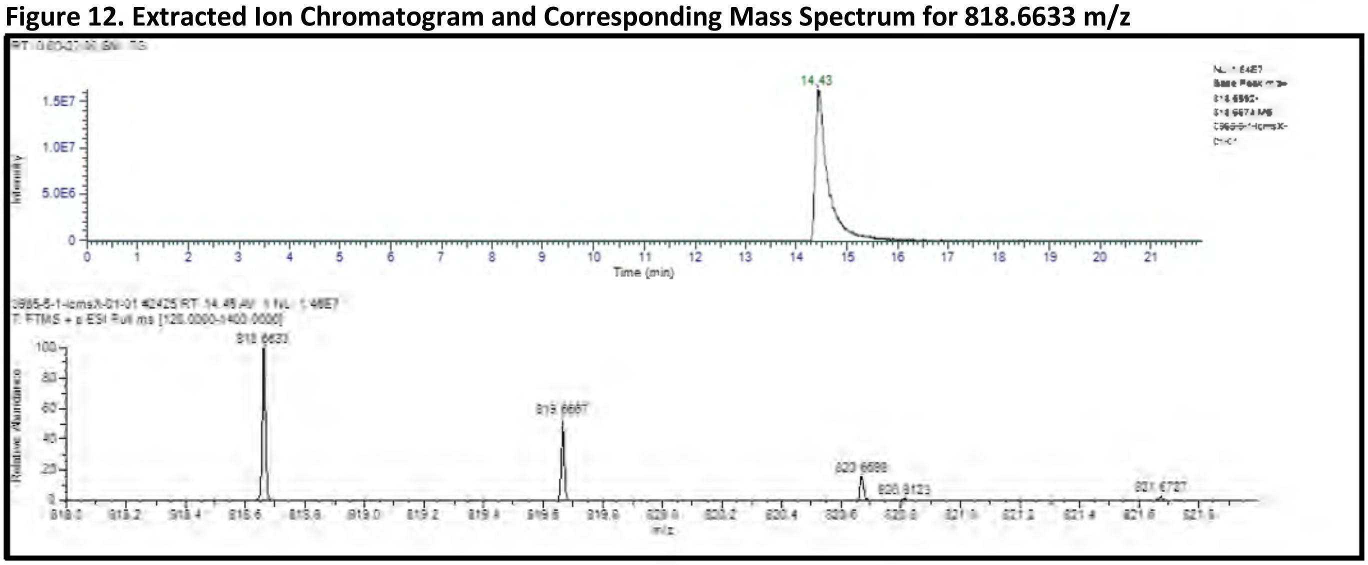
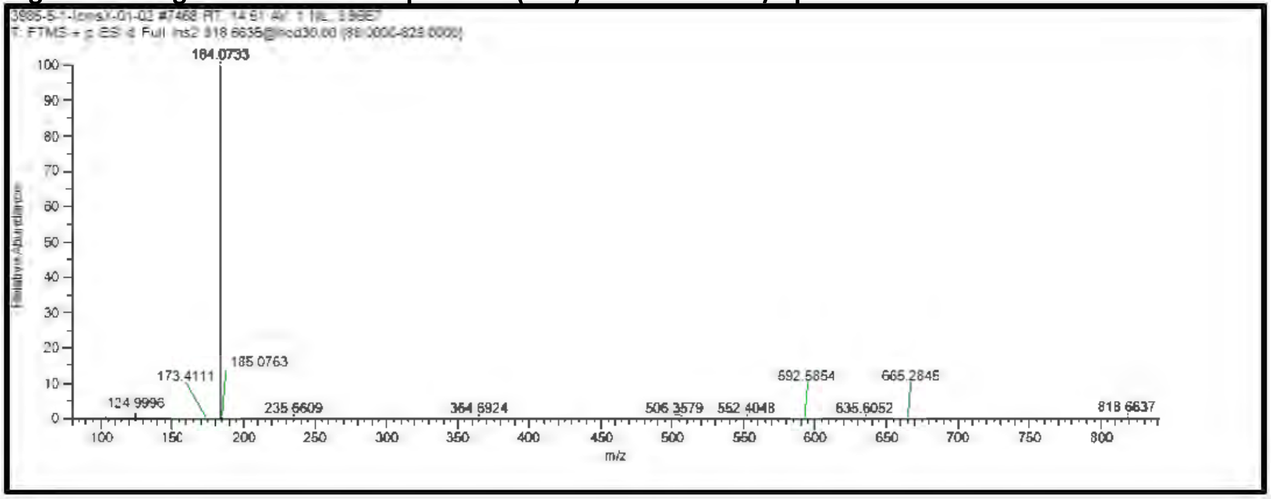


Figure 13. Fragmentation Mass Spectrum (MS^2) of 818.6633 m/z precursor ion



To further support the assignments of 1-palmitoyl-2-stearoyl-sn-glycerol-3-phosphocholine (16:0-18:0) and 1-stearoyl-2-arachidoyl-sn-glycerol-3-phosphocholine (18:0-20:0) in S2, standards of 1-palmitoyl-2-stearoyl-sn-glycerol-3-phosphocholine (PSPC, 16:0-18:0 PC) (Avanti, PN: 850456P), 1,2-diheptadecanoyl-sn-glycero-3-phosphocholine (17:0 PC) (Avanti, PN: 850360P), and 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine (19:0 PC) (Avanti, PN: 850367P) were purchased. A standard of 1-stearoyl-2-arachidoyl-sn-glycerol-3-phosphocholine (18:0-20:0) was not commercially available. For confirmation of the two proposed structures Sample S2 and prepared standards were analyzed by LC/MS

Figure 14 shows a stacked comparison of extracted ion chromatograms (762.6010 m/z) for Sample S2 and the 17:0 PC and 16:0-18:0 PC standards. The retention times match and are what would be expected from elution on a C30 column in comparison to DSPC; having two less carbons would lead to a shorter retention time due to being less non-polar. A stacked comparison of mass spectra (MS^1) for Sample S2, PSPC (16:0-18:0 PC standard), and the simulated isotope distribution for $C_{42}H_{84}NO_8P$ is shown in Figure 15. The agreement in retention time and mass spectra supports the assignment of 16:0-18:0 PC for the 762.6010 m/z in S2. Further experiments were needed to confirm the fatty acid chain.

Figure 14. Sample S2 and Standard Comparison – Positive Mode Extracted Ion Chromatogram 762.6010 m/z

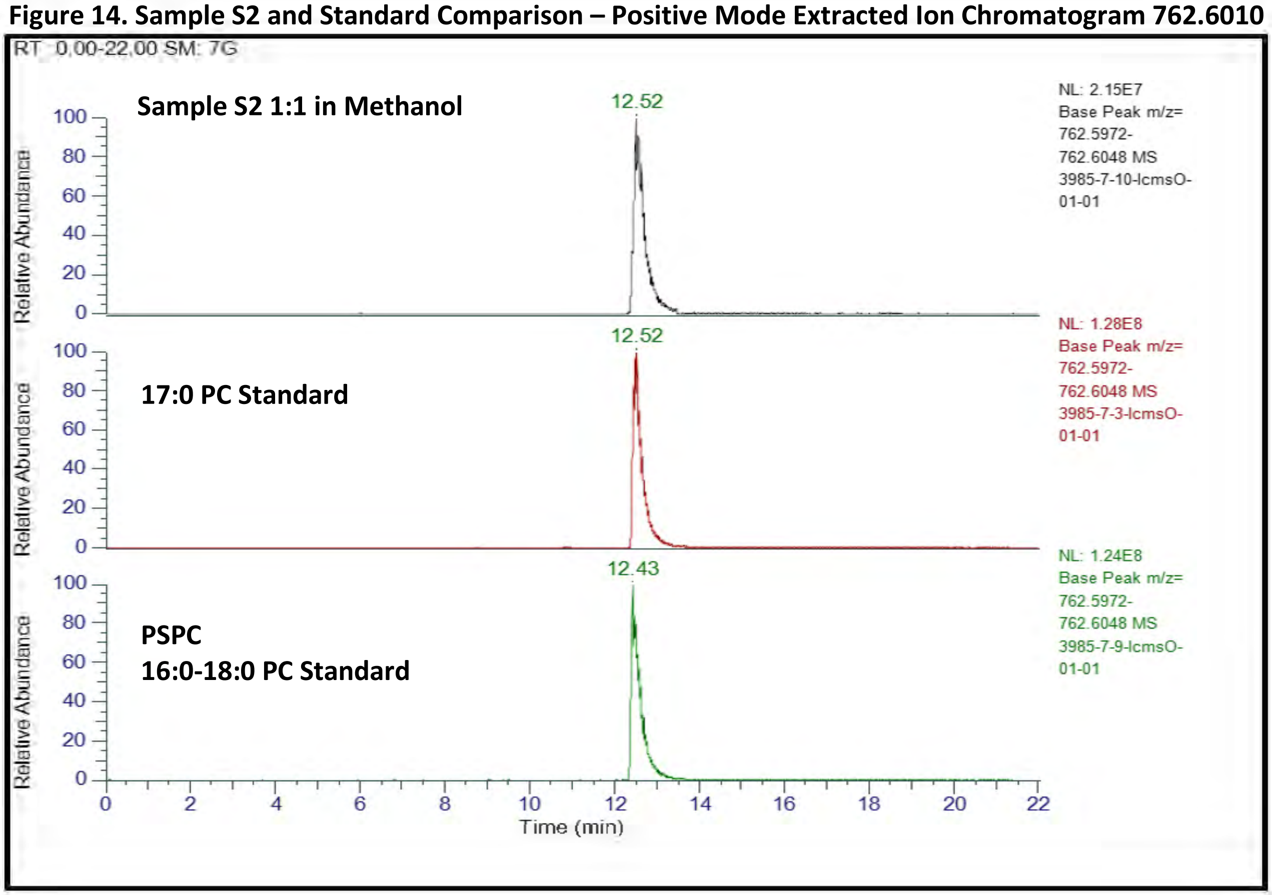


Figure 15. Mass Spectral Comparison (MS^1) of Sample, Standard and Simulated Spectrum

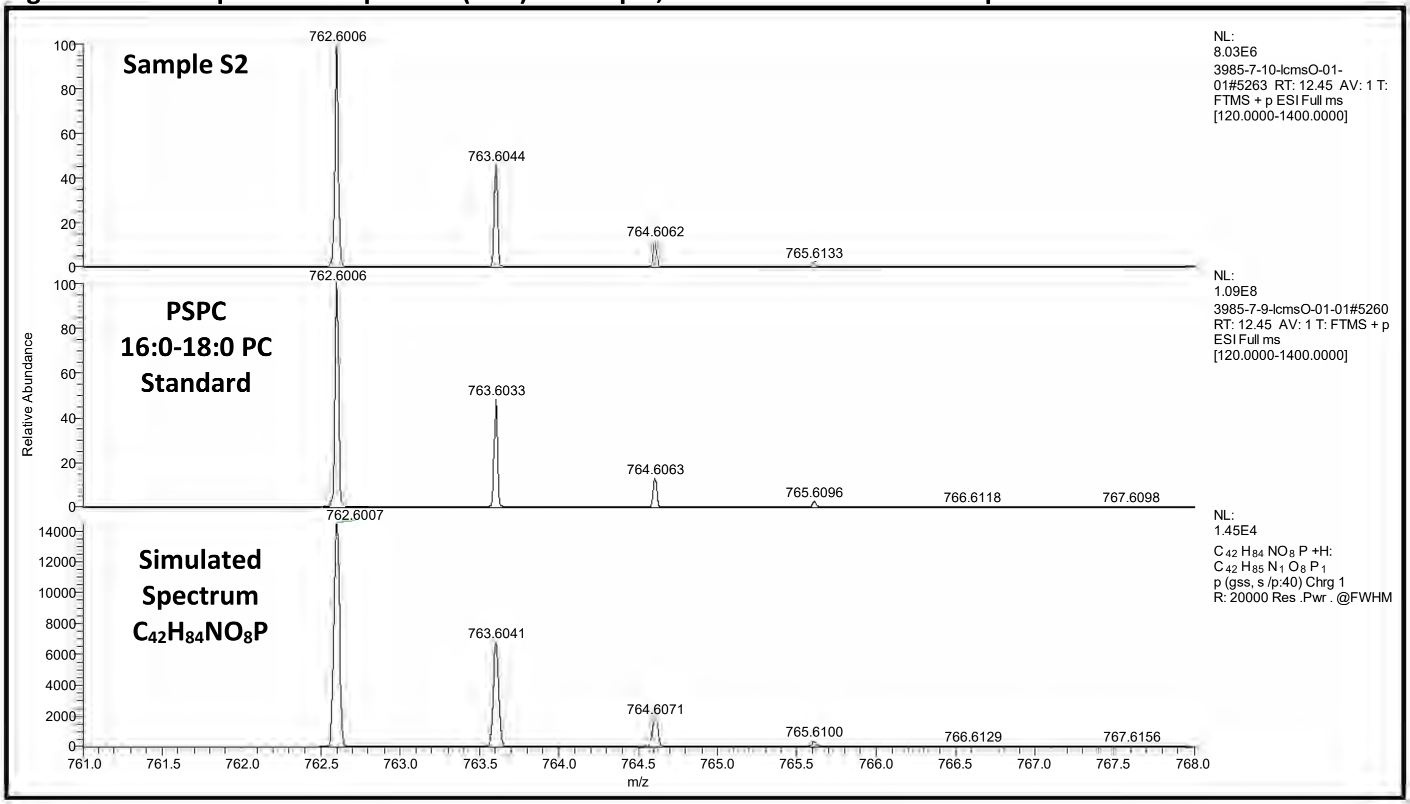


Figure 16 shows a stacked comparison of extracted ion chromatograms (818.6630 m/z) for Sample S2 and the 19:0 PC standards. The retention times match and are what would be expected from elution on a C30 column in comparison to DSPC; having two more carbons would lead to a longer retention time due to being more non-polar. A stacked comparison of mass spectra (MS^1) for Sample S2 and the simulated isotope distribution for $C_{46}H_{92}NO_8P$ is shown in Figure 17. The agreement in retention time with 19:0 PC and mass spectra support the assignment of 18:0-20:0 PC for the 818.6630 m/z in S2. Further experiments were needed to confirm the fatty acid chain.

Figure 16. Sample S2 and Standard Comparison – Positive Mode Extracted Ion Chromatogram 818.6630 m/z

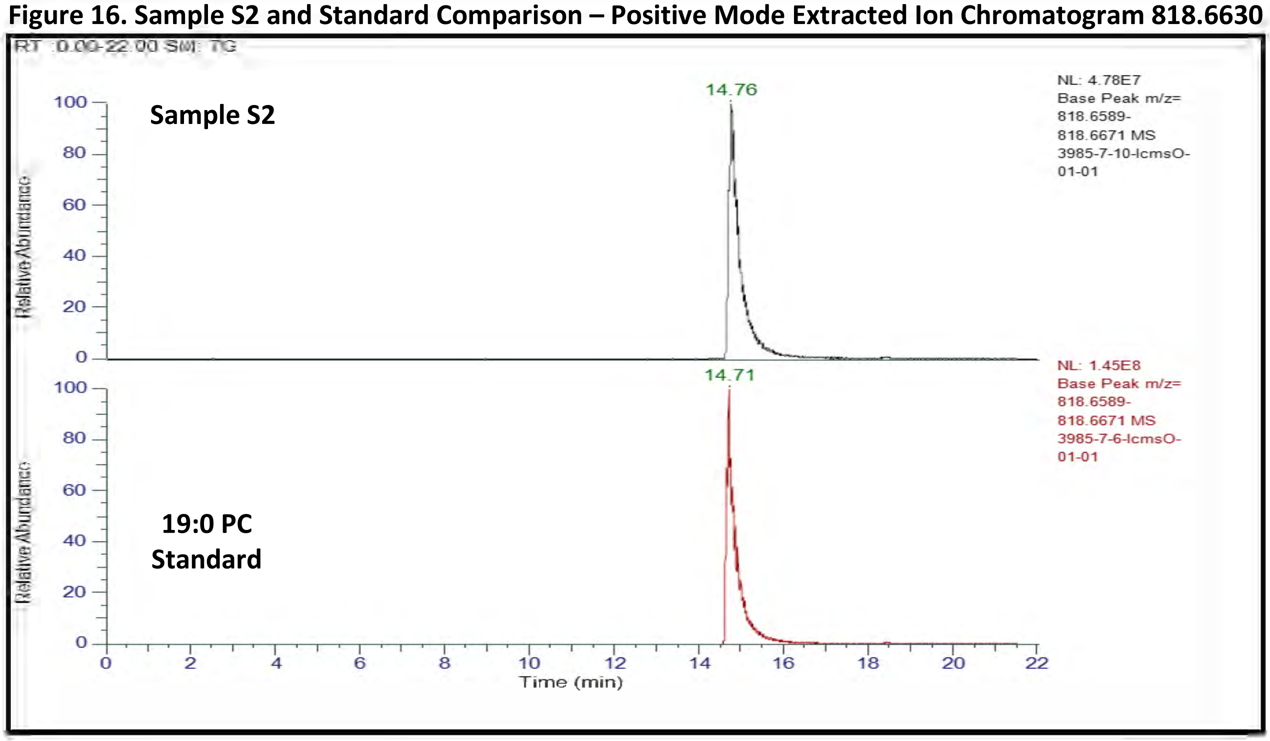
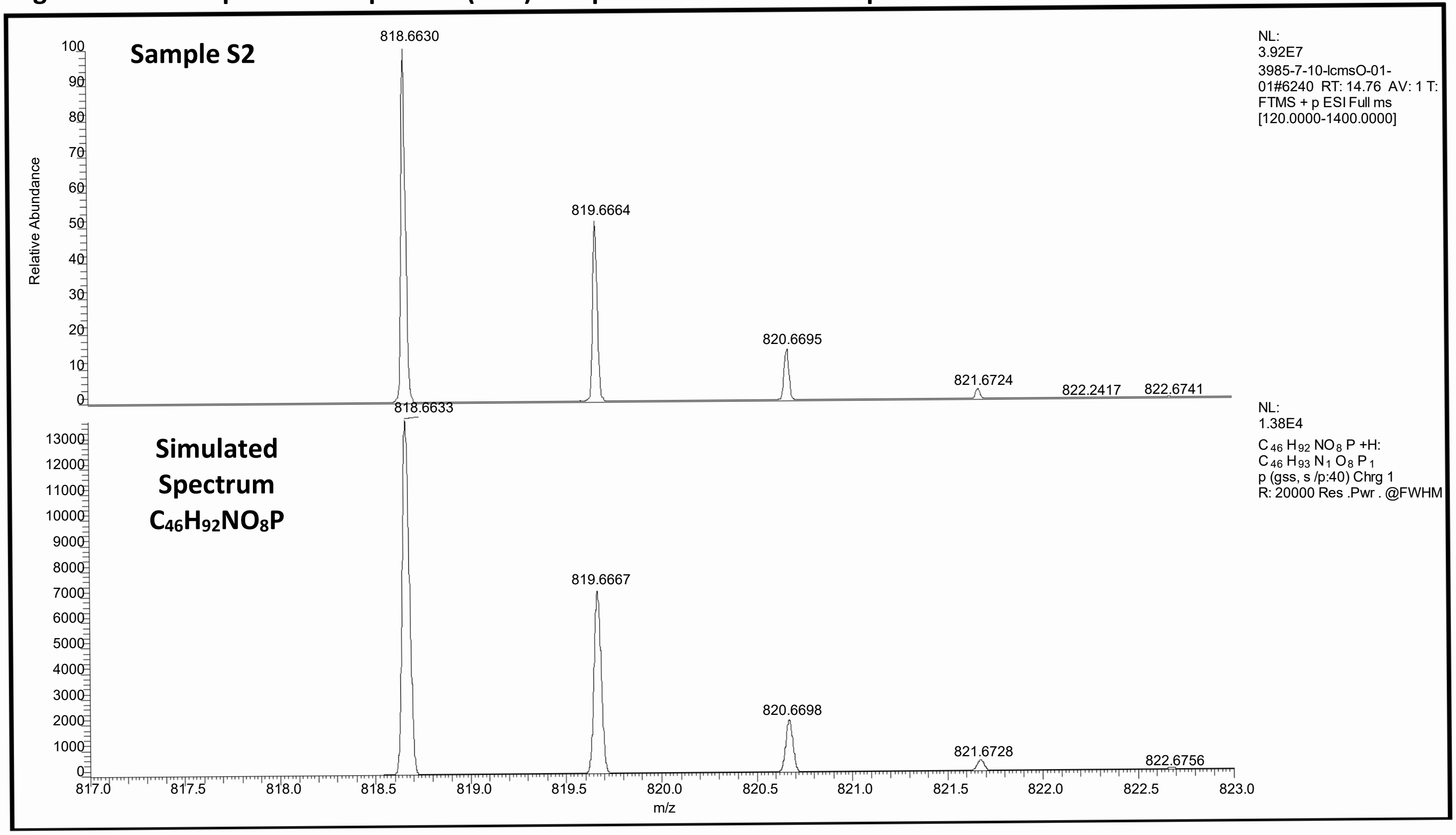


Figure 17. Mass Spectral Comparison (MS[1]) Sample S2 and Simulated Spectrum



In addition to forming an $[M+H]^+$ adduct in positive mode, neutral phospholipids can form an adduct with the mobile phase additive in negative mode. In this case, an $[M+DFA-H]^-$ adduct is observed where $C_2HF_2O_2^-$ is added to the neutral formula and $[M+94.9950]^-$ is observed. When these negative mode adducts are fragmented by HCD activation, the fatty acid esters are cleaved to give negative mode fragment ions for the fatty acids present in the molecule.[6] The bonds that are cleaved and the resulting fragment ions for DSPC, PSPC (16:0-18:0 PC), and 18:0-20:0 PC are shown in Figure 18 through Figure 20. The fragment ions and formulas for the observed fatty acid chain lengths are summarized in Table 3.

Figure 18. DSPC Structure Showing Fatty Acid Chains

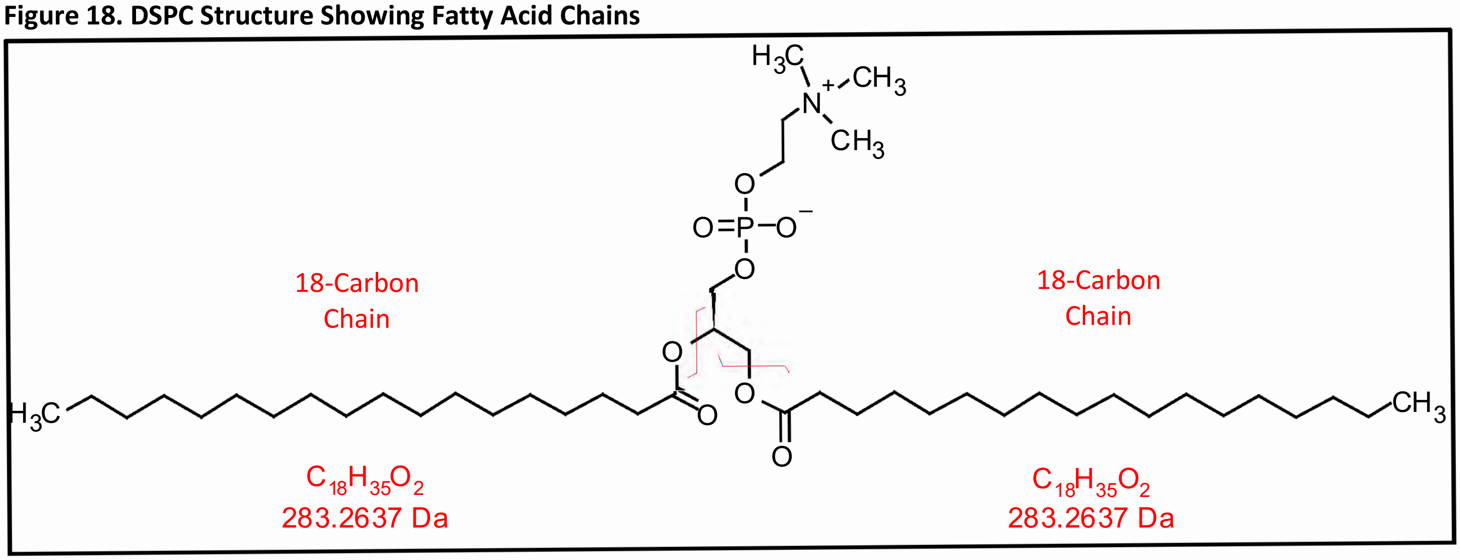


Figure 19. 1-palmitoyl-2-stearoyl-sn-glycerol-3-phosphocholine (PSPC, 16:0-18:0 PC) Structure Showing Fatty Acid Chains

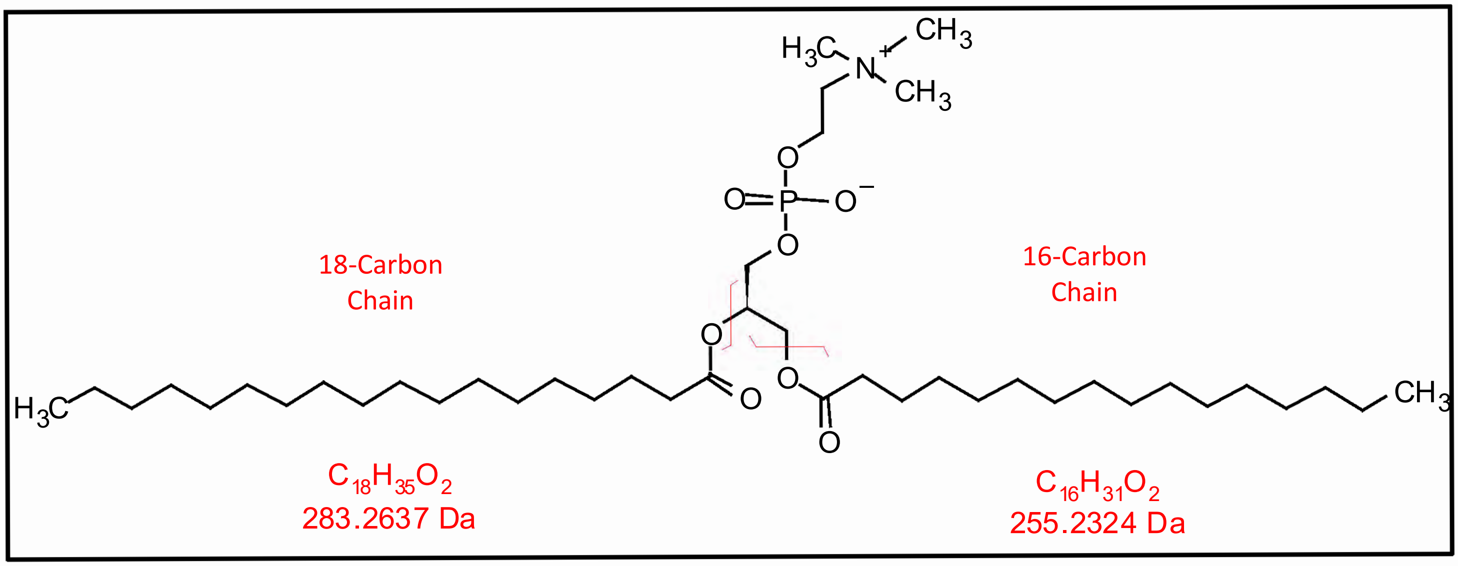


Figure 20. 1-stearoyl-2-arachidoyl-sn-glycerol-3-phosphocholine (18:0-20:0 PC) Structure Showing Fatty Acid Chains

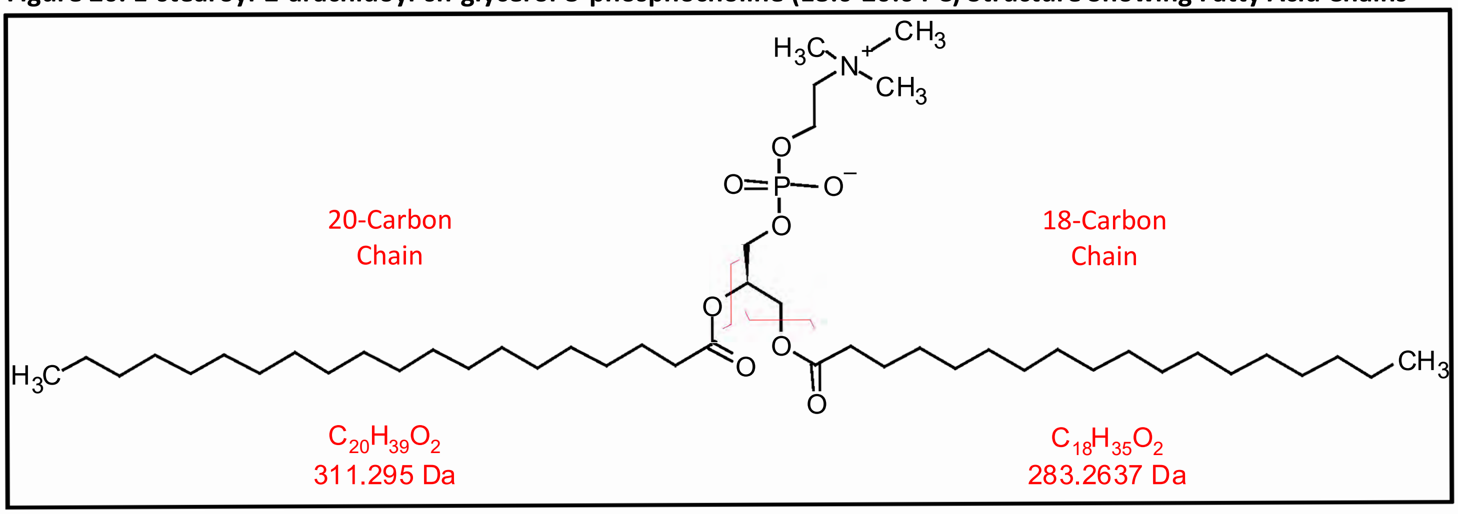


Table 3. Fatty Acid Chain Lengths and their resulting fragment ions

Fatty Acid Chain Length	Molecular Formula	Fragment Ion m/z
16	$C_{16}H_{31}O_2$	255.2324
18	$C_{18}H_{35}O_2$	283.2637
20	$C_{20}H_{39}O_2$	311.2950

To identify the fatty acids and confirm the assignments, the standards and sample were analyzed in negative mode with HCD fragmentation. A stacked comparison of negative mode EIC's (856.5878 m/z) for the Sample S2, 17:0 PC standard, and PSPC (16:0-18:0 PC) standard are shown in Figure 21. A stacked comparison of negative mode EIC's (912.6504 m/z) for the Sample S2 and 19:0 PC standard are shown in Figure 22. The retention times are still in agreement in negative mode. The MS^2 of DSPC is shown in Figure 23 and the expected fragment ion (283.2640 m/z) for stearic acid is observed. The MS^2 spectra of 856.588 m/z in S2 and the 16:0-18:0 PC standard are shown in Figure 24 and Figure 25. The match of the 255.2326 m/z and 283.2638 m/z ions confirms the PSPC (16:0-18:0 PC) assignment in the sample. The MS^2 spectra of 912.6504 m/z in S2 and the 19:0 PC standard are shown in Figure 26 and Figure 27. The 311.2955 m/z and 283.2638 m/z ions resulting from the sample fragmentation do not match with the 297.2797 m/z ion of the 19:0 PC standard. This result supports the assignment of the of 18:0-20:0 PC as the structure for the peak with formula $C_{46}H_{92}NO_8P$ (818.6633 m/z in positive mode). The MS^2 of 912.6504 m/z in S2 compares favorably with the in silico MS^2 for 18:0-20:0 PC from the

LipidBlast database.[7] The in silico MS^2 spectrum for 18:0-20:0 PC, reproduced from the MassBank of North America, both zoomed and full scale is shown in Figure 28 and Figure 29.[8]

Figure 21. Sample S2 and Standard Comparison - Negative Mode Extracted Ion Chromatogram 856.5878 m/z

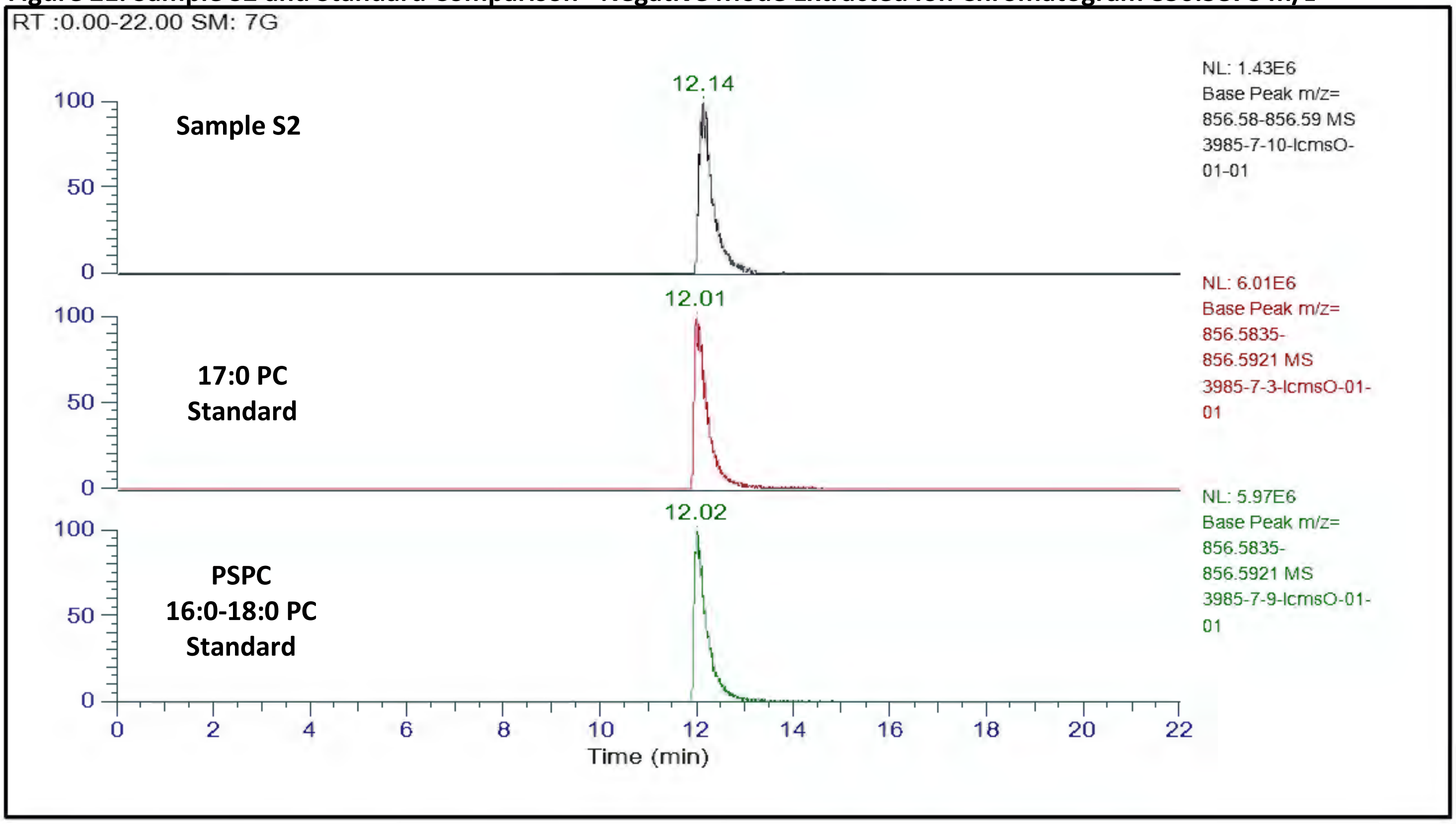


Figure 22. Sample S2 and Standard Comparison - Negative Mode Extracted Ion Chromatogram 912.6504 m/z

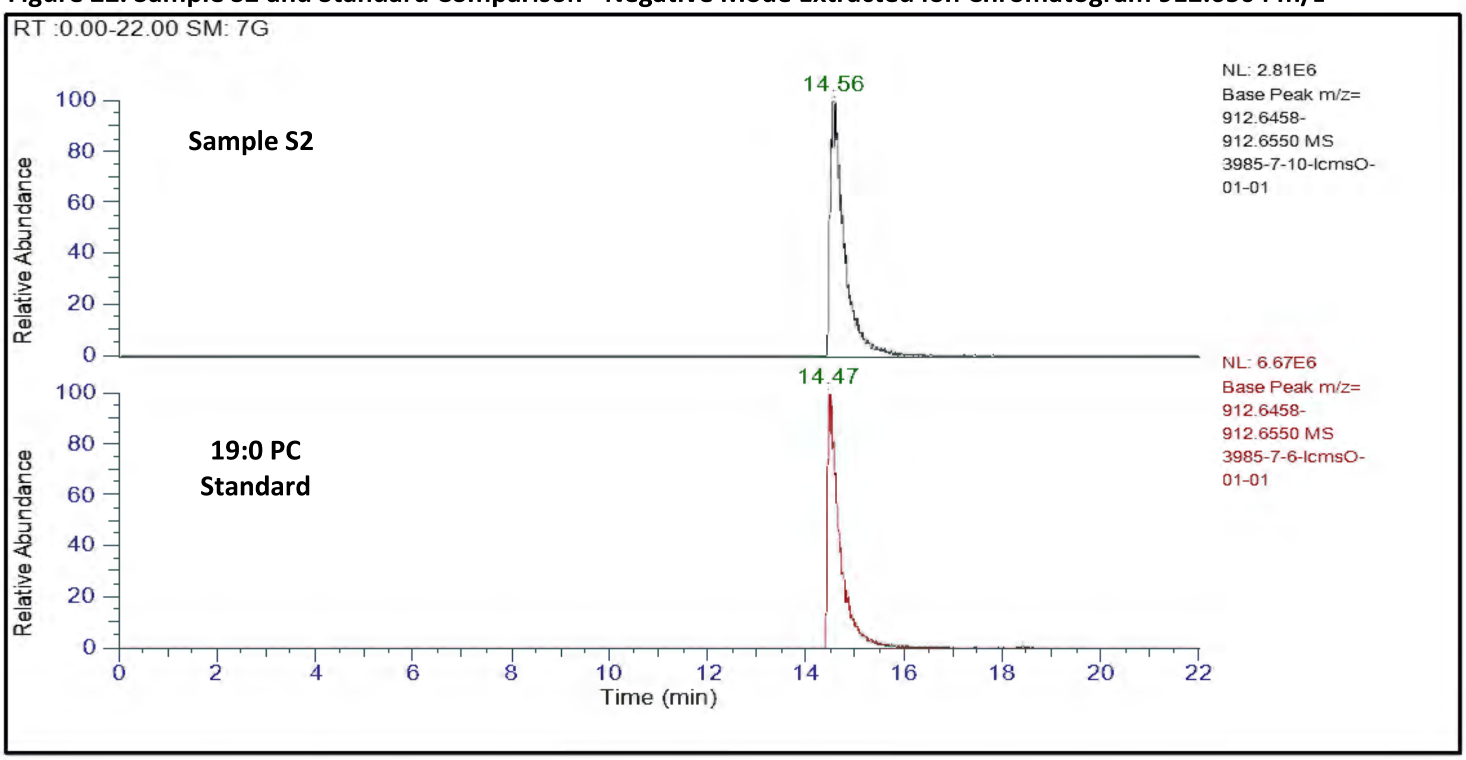


Figure 23. DSPC Fragmentation Mass Spectra of 884.6194 m/z precursor ion

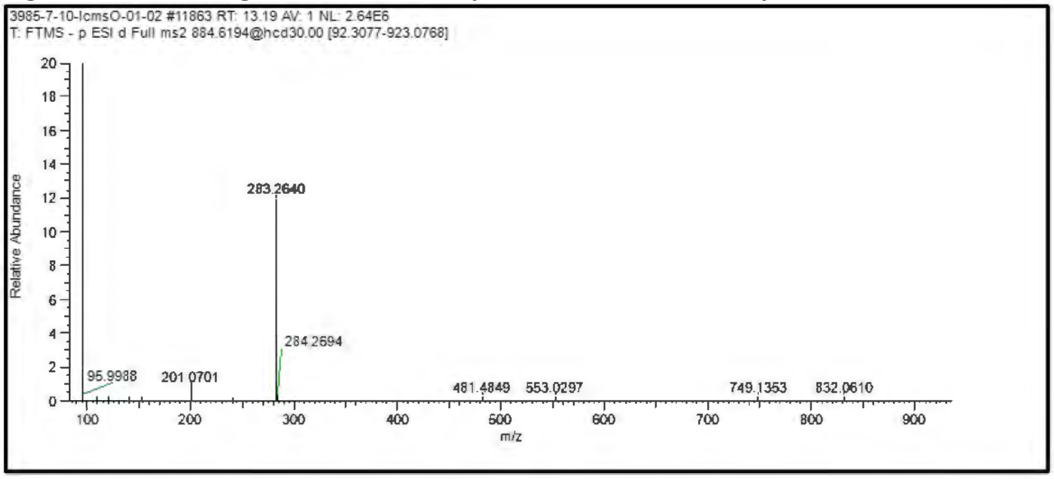


Figure 24. Sample S2 and Standard Comparison of Fragmentation Spectra 856.5881 m/z precursor ion

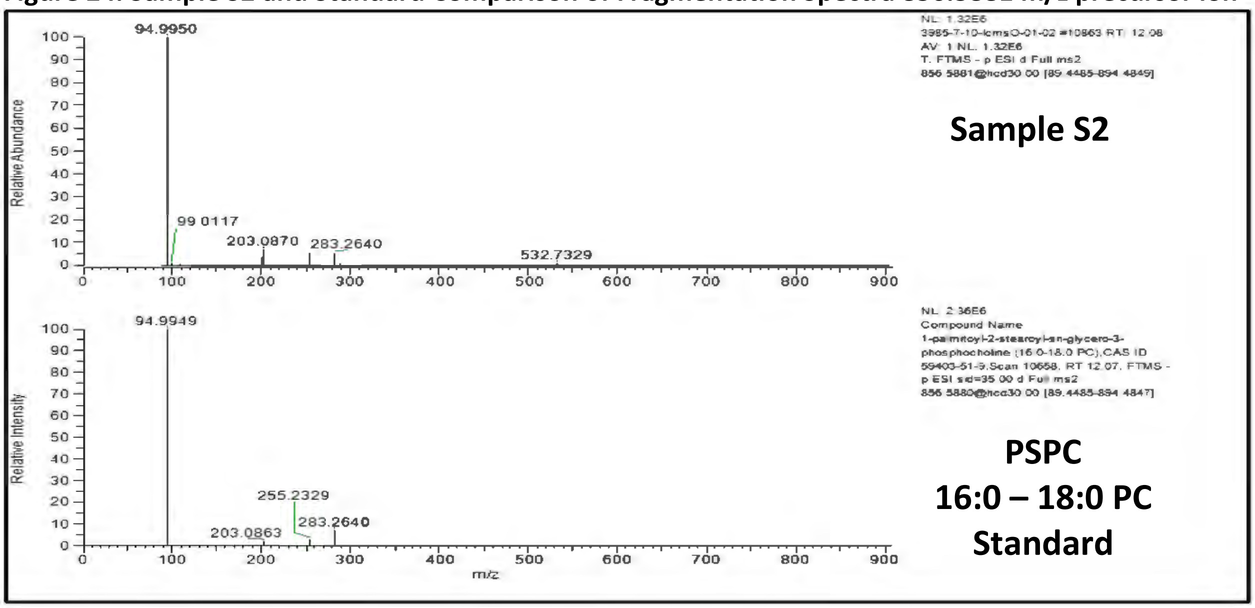


Figure 25. Sample S2 and Standard Comparison of Fragmentation Spectra 856.5881 m/z precursor ion (zoom)

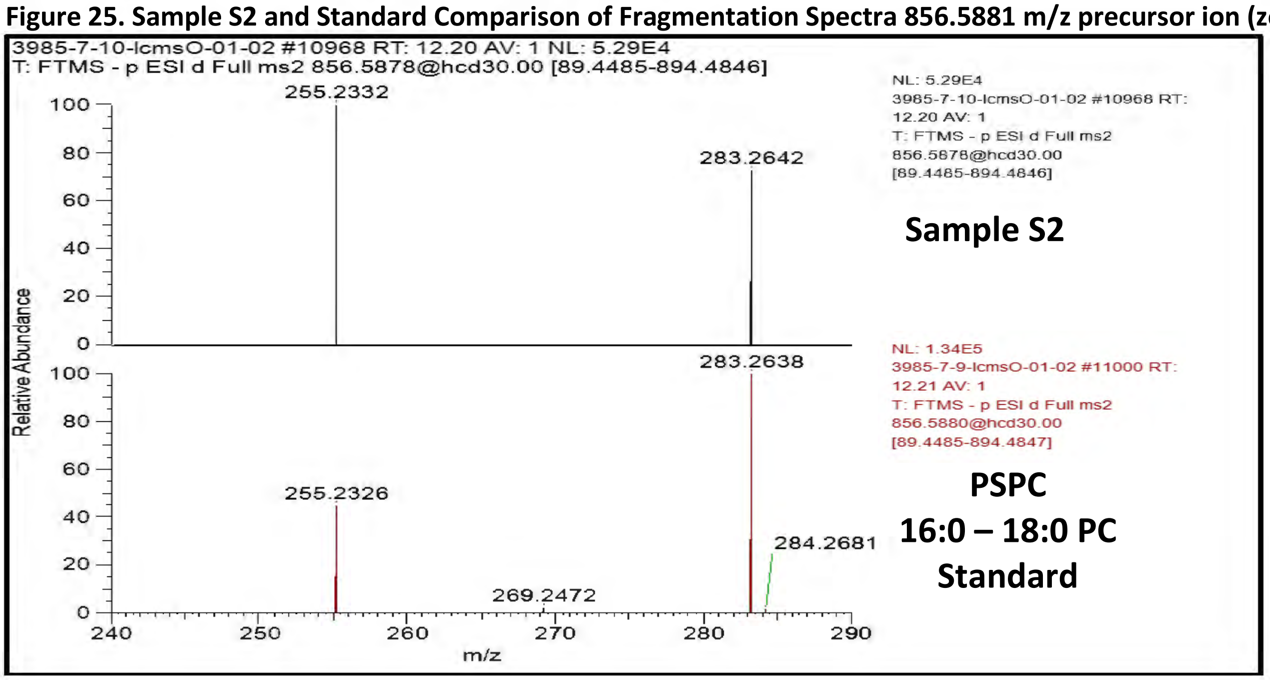


Figure 26. Sample S2 and Standard Comparison of Fragmentation Spectra 912.6505 m/z precursor ion

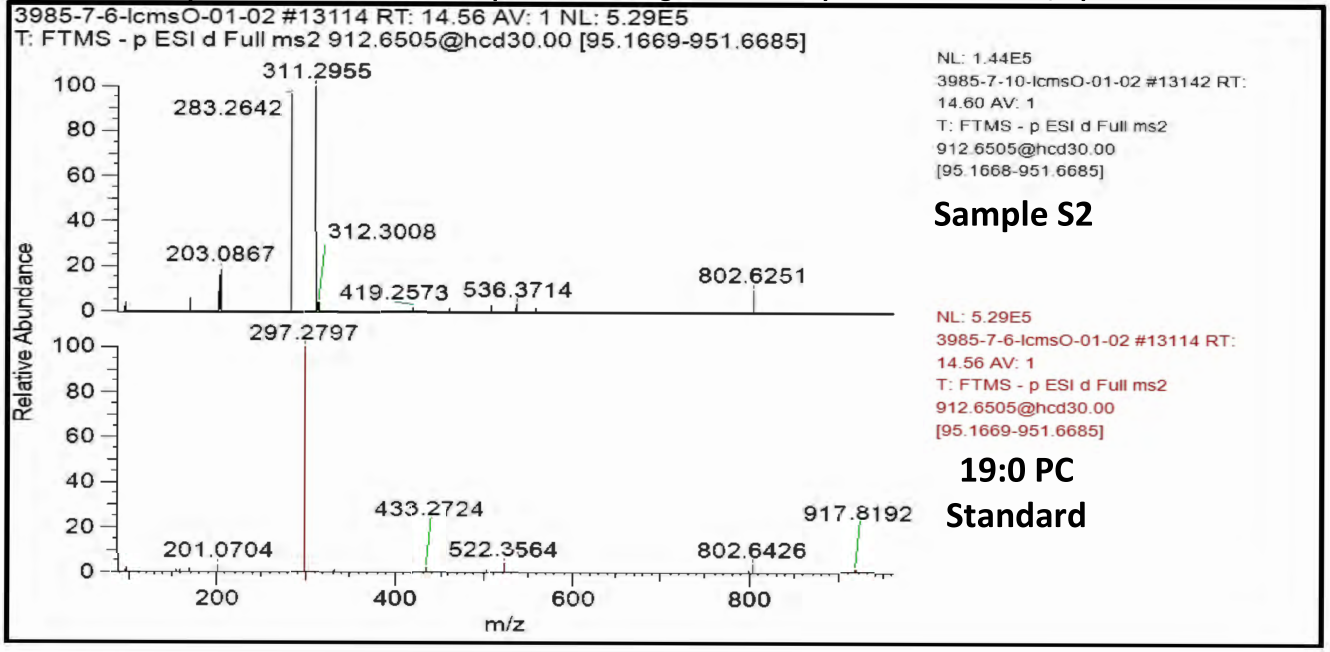


Figure 27. Sample S2 and Standard Comparison of Fragmentation Spectra 912.6505 m/z precursor ion (zoom)

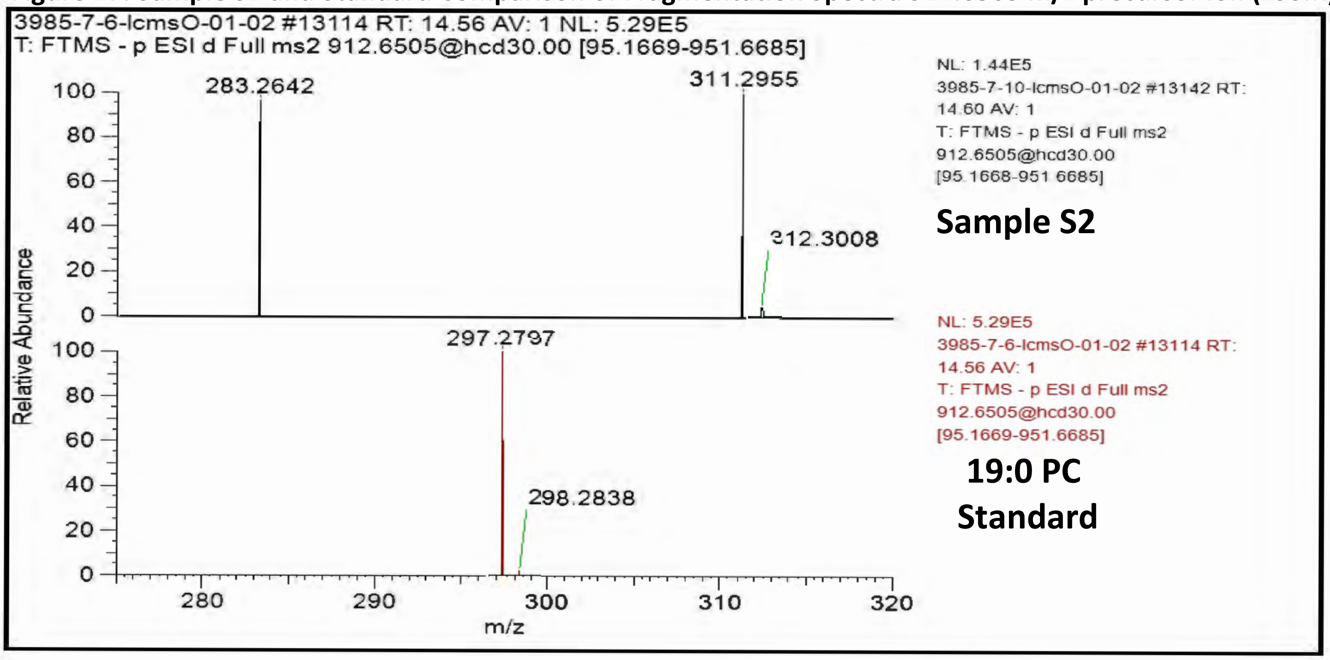


Figure 28. In-Silico Spectrum of 18:0-20:0 PC from MassBank of North America (zoom)

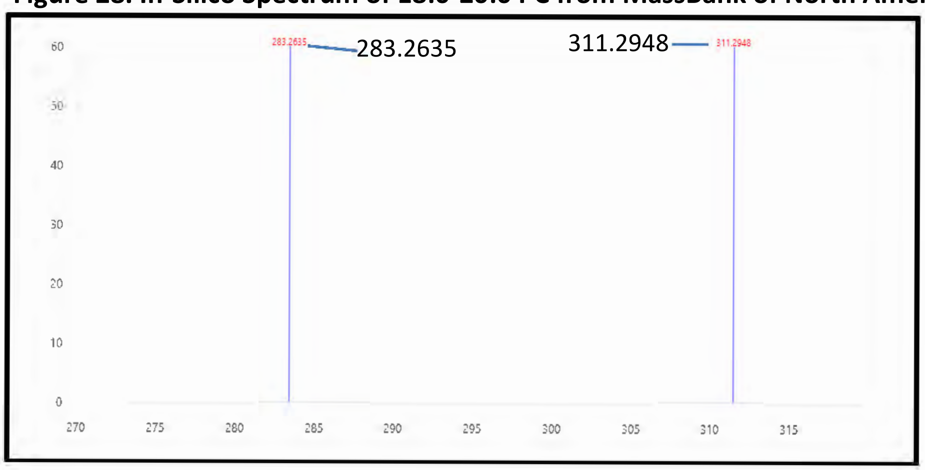
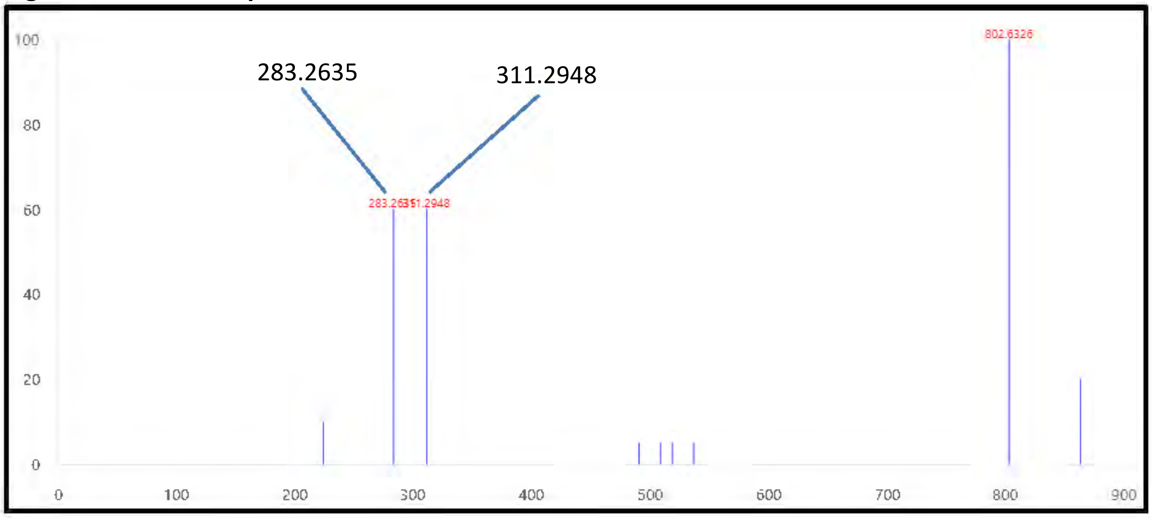


Figure 29. In-Silico Spectrum of 18:0-20:0 PC from MassBank of North America



CONCLUSIONS

Onpattro (Patisiran) Lipid Complex Injection (S2, 10 mg/5 mL) was screened for neutral phospholipids by LC/MS. In addition to DSPC and Lyso PC, the next two major neutral phospholipids were identified as PSPC (16:0-18:0 PC, CAS# 59403-51-9) and 18:0-20:0 PC (CAS# 61574-10-5) with the structures shown in Figure 30 and Figure 31. The positive mode LC/MS data supports the assignment of the molecular formulas and the presence of the phosphocholine moiety in each structure. The negative mode LC/MS data supports the assignment of the fatty acids present in the neutral phospholipids based on the MS^2 fragment ions.

Figure 30. Structure and Formula for PSPC (16:0-18:0 PC) in S2

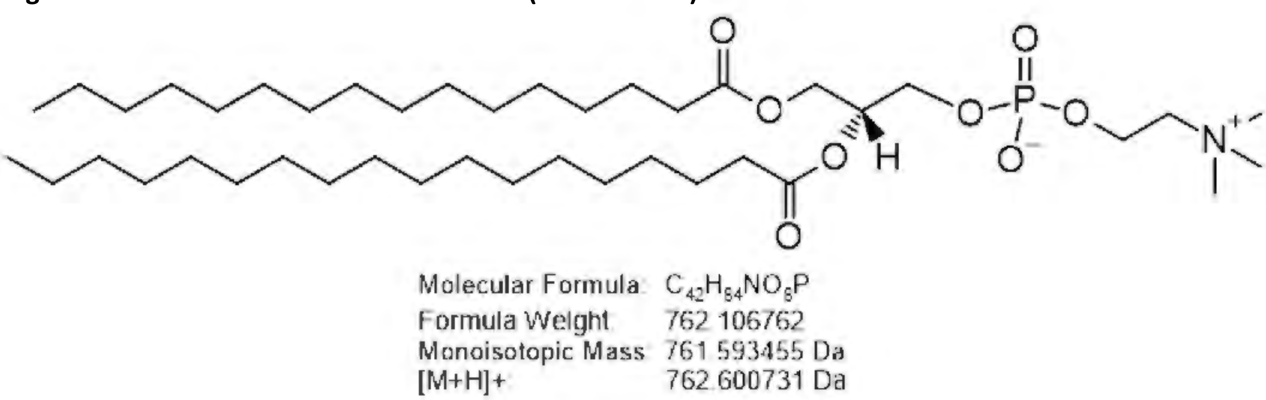
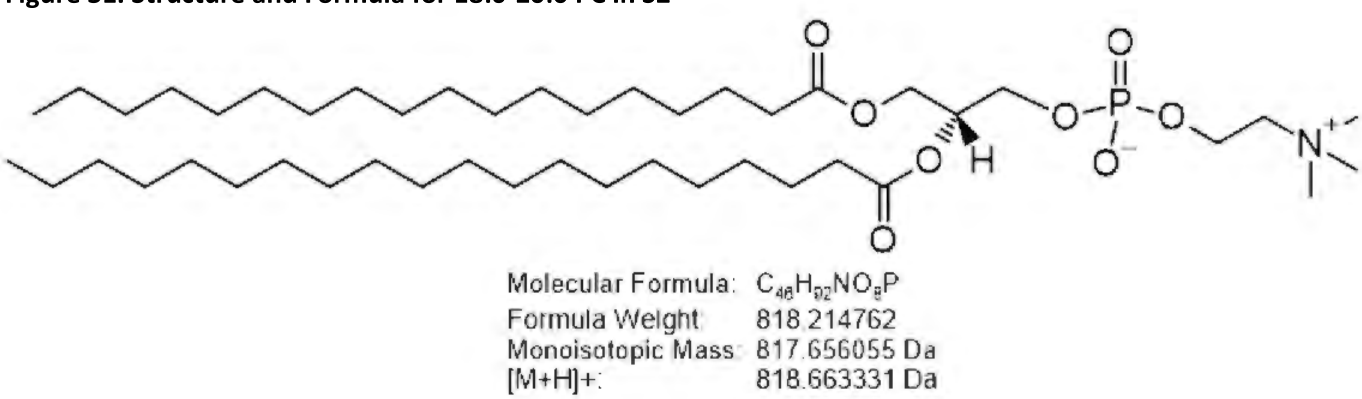


Figure 31. Structure and Formula for 18:0-20:0 PC in S2



LABORATORY CERTIFIED TO ISO 17025	ADDRESS	ISO 17025 CERTIFICATION NUMBER
CA	810 Kifer Road Sunnyvale, CA 94086	2797.01
MN	18705 Lake Drive East Chanhassen, MN 55317	2797.02
NY	103 Commerce Blvd Liverpool, NY 13088	2797.03
China	1F, Building 4 No. 1151 Lianxi Road Pudong Area, Shanghai, 201204	2797.04
NJ	104 East Windsor Drive, Suite 101 East Windsor, NJ 08520	2797.05
LA	250 North Nash Street El Segundo, CA 90245	2797.07

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Exhibit D

comment

The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs

The regulatory approval of Onpattro, a lipid nanoparticle-based short interfering RNA drug for the treatment of polyneuropathies induced by hereditary transthyretin amyloidosis, paves the way for clinical development of many nucleic acid-based therapies enabled by nanoparticle delivery.

Akin Akinc, Martin A. Maier, Muthiah Manoharan, Kevin Fitzgerald, Muthusamy Jayaraman, Scott Barros, Steven Ansell, Xinyao Du, Michael J. Hope, Thomas D. Madden, Barbara L. Mui, Sean C. Semple, Ying K. Tam, Marco Ciufolini, Dominik Witzigmann, Jayesh A. Kulkarni, Roy van der Meel and Pieter R. Cullis

Nanomedicines resulting from the application of nanotechnology to medicine are having an increasing impact on the treatment of disease. This applies particularly to nanomedicines using lipid nanoparticle (LNP) drug delivery systems as there are now more than ten US Food and Drug Administration (FDA) approved pharmaceuticals employing LNPs to deliver drugs to disease sites (Table 1). Most of these nanomedicines are formulations of cancer drugs that offer the benefits of reduced toxicity and/or enhanced efficacy compared to the 'free' drug[1]. Due to the clinical success of LNP-based drug delivery systems, we now have a good understanding of the requirements for successful clinical translation of LNP systems for delivery of small molecules. Translational criteria include a size range of 100 nm or less, highly efficient encapsulation techniques, a low surface charge, robust, scalable manufacturing processes and adequate product stability[2].

It is of great interest to extend LNP technology to delivery of nucleic acid-based drugs, such as short interfering RNA (siRNA), messenger RNA (mRNA) and gene editing constructs. Unmodified nucleic acid-based drugs face particular delivery problems, because they are readily broken down in biological fluids, do not accumulate in target tissues and cannot penetrate into target cells even if they get to the desired tissues. Unfortunately, many of the techniques developed for generating clinically viable LNP formulations of small molecule drugs cannot be applied to nucleic acid polymers owing to their large size and negative charge. Further, LNP formulations of small molecule drugs have only to release drug cargo after arrival in the target tissue; by contrast, LNP formulations of nucleic acid-based drugs must also facilitate intracellular delivery of these macromolecules into target cells.

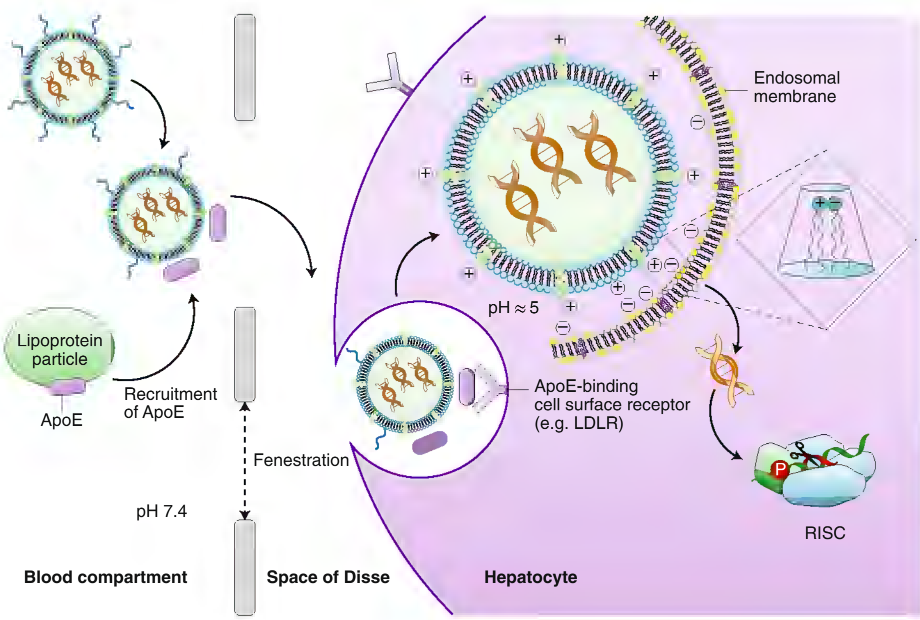

Fig. 1 | Integrated model of lipid nanoparticle (LNP)-mediated delivery of siRNA to hepatocytes in vivo. Key steps include the dissociation of PEG-lipids from the particle surface, recruitment of endogenous ApoE to the LNP surface, trafficking of LNPs through fenestrated endothelium and binding to low density lipoprotein receptors and other ApoE-binding receptors on hepatocytes, internalization of LNPs via endocytosis, protonation of the ionizable lipid due to the low pH in the endosome, interaction of the protonated ionizable lipid with negatively charged endogenous lipids, which results in the destabilization of the endosomal membrane, and release of siRNA into the cytoplasm, where it can engage with the RNAi machinery. RISC, RNA-induced silencing complex. LDLR, low density lipoprotein receptor.

Here, we describe the successful preclinical development and clinical translation of patisiran (trade name Onpattro), which is an LNP formulation of siRNA for the treatment of polyneuropathies resulting from the hereditary disease transthyretin-mediated amyloidosis (hATTR). This drug acts by inhibiting the synthesis of the transthyretin (TTR) protein in the liver. The positive results of a global phase 3 study[3] resulted in FDA approval of Onpattro in August 2018. The success of

Table 1 | LNP drugs that have received regulatory approval from the FDA or EMA

Name	Encapsulated drug	Indication	Year approved	Company
AmBisome	Amphotericin B	Fungal infections Leishmaniasis	1990 (Europe) 1997 (USA)	Gilead
Doxil/Caelyx	Doxorubicin	Kaposi's sarcoma Ovarian cancer Breast Cancer	1995 (USA) 1999 (USA) 2003 (Europe)	Johnson& Johnson
DaunoXome	Daunorubicin	Kaposi's sarcoma	1996 (Europe), 1996 (USA)	Galen
Myocet	Doxorubicin	Breast cancer	2000 (Europe)	Cephalon
Abelcet	Amphotericin B	Aspergillosis	1995 (USA)	Enzon
Amphotec	Amphotericin B	Invasive aspergillosis	1996 (USA)	Intermune
Visudyne	Verteporfin	Wet macular degeneration	2000 (USA)	QLT
Marqibo	Vincristine	Acute lymphoblastic leukemia	2012 (USA)	Spectrum Pharma
Onyvide	Irinotecan	Metastatic pancreatic cancer	2015 (USA)	Ipsen Biopharma
Vyxeos	Daunorubicin, Cytarabine	Acute lymphocytic leukemia	2017 (USA)	Jazz Pharma
Onpattro	siRNA targeting transthyretin	Transthyretin induced amyloidosis (hATTR)	2018 (USA), 2018 (Europe)	Alnylam Pharmaceuticals

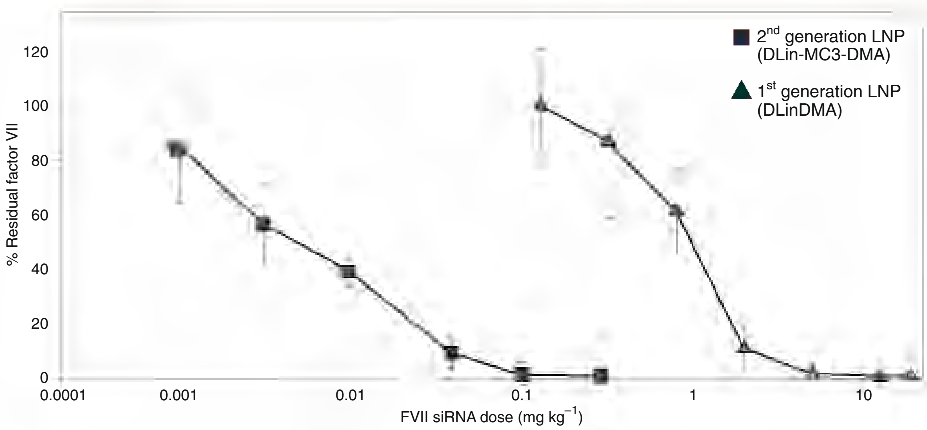

Fig. 2 | LNP siRNA systems containing 2nd generation ionizable aminolipids exhibit greatly improved potency for silencing factor VII (FVII) in the liver. The data presented shows dose-dependent silencing of FVII following i.v. injection of LNP encapsulating siRNA against FVII in a mouse model. 2nd generation LNP containing heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate (DLin-MC3-DMA) are more than two orders of magnitude more potent than 1st generation LNP containing 1,2-dilinoleyl-*N*,*N*-dimethyl-3-aminopropane (DLinDMA).

Onpattro heralds the arrival of a new class of medicines based on nucleic acid polymers. In particular, subsequent studies of closely related LNP systems containing much larger mRNA cargos indicate that LNP delivery technology can potentially enable most forms of nucleic acid-based therapies.

Preclinical development

The basic features required of an LNP siRNA system with the potential for clinical translation include efficient encapsulation of siRNA into an LNP with low surface charge, a diameter of 100 nm or less and the ability to deliver encapsulated siRNA to the cytoplasm of hepatocytes in vivo following intravenous administration. With regard to encapsulation, nucleic acid polymers can be readily associated with lipidic particles containing permanently positively charged lipids; however, such positively charged systems induce pronounced toxicity in vivo due to immune activation (activation of complement and coagulation pathways as well as cytokine stimulation) and cytotoxicity. To circumvent this problem, we developed ionizable cationic lipids that possess an amine function with an acid dissociation constant (pKa) of ~6.5 (ref. [4]). These lipids are positively charged at acidic pH values, but nearly neutral at physiological pH. Efficient encapsulation of siRNA into LNPs can then be achieved by rapid mixing of lipids in ethanol with siRNA in aqueous media at low pH (pH 4) using a readily scalable manufacturing process. These LNP systems, which have a novel 'solid core' structure[5], display low surface charge at physiological pH and are relatively non-toxic and non-immunogenic.

Diameters of 100 nm or less could be achieved by incorporating polyethylene glycol (PEG)-lipids that associate with the surface of the LNP. LNP size can then be regulated by adjusting the proportion of surface PEG lipid to core lipid to generate sizes over the range 20–100 nm[6]. The presence of a PEG coating on the LNP surface has the disadvantage of inhibiting interactions with target cells and thus reducing intracellular delivery. This problem was overcome by using PEG-lipids with relatively short C_{14} acyl chains. Such PEG-lipids remain associated with the particles during formulation and under storage conditions; however, in the presence of a lipid sink (for example, lipoprotein particles in plasma), the PEG-lipids can exchange out of the LNP, thereby generating an unshielded particle that can engage with target cells to enable uptake[7].

The development of LNP siRNA systems with high loading efficiencies, defined size and low surface charge satisfied the basic criteria for clinical potential; however, the potency of these systems for gene silencing in hepatocytes remained to be characterized and optimized. As the in vitro potency of an LNP nanomedicine rarely correlates with in vivo performance, we moved directly to an in vivo model to optimize gene silencing properties. LNPs containing siRNA against factor VII (FVII) were administered to mice

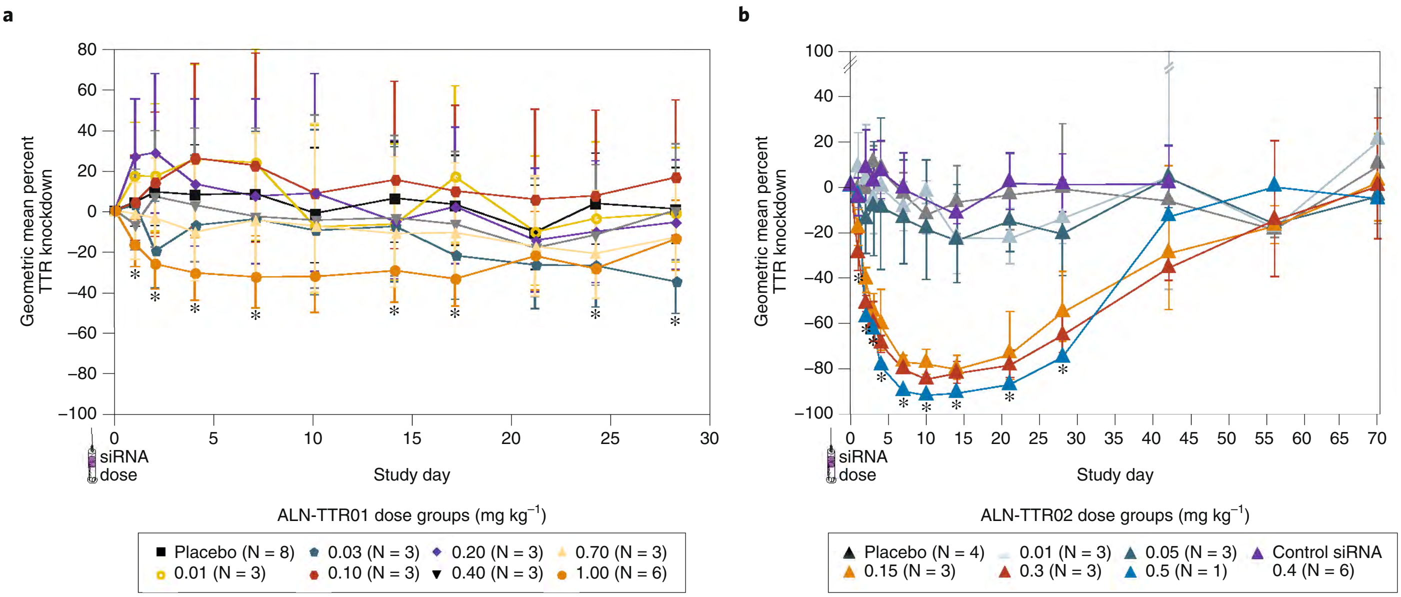

Fig. 3 | Phase I clinical trials of ALN-TTR01 and ALN-TTR02 (patisiran). a,b, Mean percent serum transthyretin (TTR) knockdown at the indicated time points, as compared with the baseline in groups of patients receiving either placebo or increasing doses of ALN-TTR01 (a) and in healthy subjects receiving either placebo or increasing doses of ALN-TTR02 (patisiran) (b). The error bars indicate 95% confidence intervals. Data from ref. [13].

to silence the FVII gene in hepatocytes, providing a convenient assay to optimize gene silencing potency in vivo. Initial work showed that LNP siRNA systems containing the ionizable lipid 1,2-dilinoleyl-*N,N*-dimethyl-3-aminopropane (DLinDMA) could silence genes in hepatocytes following intravenous (i.v.) administration[8]. However, the potency and tolerability of these LNP siRNA systems was not sufficient to warrant clinical development and a search for more active formulations commenced focusing primarily on the ionizable lipid component.

A first breakthrough was reached with the development of the ionizable lipid DLinKC2DMA[9], which substantially improved the potency and tolerability of the LNP, leading to an extensive research programme aimed at achieving ever more potent ionizable cationic lipids. The pharmacodynamics, pharmacokinetics and safety of promising formulations were evaluated in rodents, and lead candidates were then tested in non-human primates (NHPs). More than 300 ionizable lipids were designed and synthesized, leading to the identification of structure–activity relationships[10]. Notably, a remarkable dependence of LNP siRNA gene silencing potency on the acid dissociation constant (pKa) of the ionizable cationic lipid was found, with an optimum around pKa ≈ 6.4. Deviation from this pKa by as little as 0.5 units could reduce potency by 100-fold or more[10]. This pKa optimum likely reflects the required balance between a low LNP surface charge to avoid rapid clearance in the circulation and a positive charge on the ionizable lipids to enable escape out of the acidic endosome following endocytosis. Positively charged lipids interact with negatively charged lipids to disrupt bilayer membranes[11], which is a probable requirement for breaking out of endosomes and thus, for cytoplasmic delivery of siRNA.

The remarkable affinity of these LNPs for the liver, in particular for hepatocytes, was found to be facilitated by the adsorption of apolipoprotein E (ApoE) on the surface of the LNPs following i.v. administration. The particle-associated ApoE acts as a highly effective targeting ligand by binding to lipoprotein receptors on the surface of hepatocytes, thereby triggering uptake into hepatocytes by endocytosis[12]. An integrated working model of LNP-mediated delivery of siRNA was then developed to describe the key steps of the LNP journey, from the site of administration to the release of the siRNA payload into the cytoplasm of hepatocytes (Fig. 1). This improved mechanistic understanding and predictability of lipid activity enabled the discovery of increasingly potent ionizable lipids, among which heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate, later termed DLinMC3DMA (or simply MC3), exhibited an improvement in potency of more than two orders of magnitude compared to the benchmark DLinDMA formulation (Fig. 2).

After confirmation of potent TTR silencing in NHPs, the MC3 formulation containing a human TTR-targeting siRNA was transitioned into preclinical development as ALN-TTR02 (later known as patisiran). Repeat-dose toxicology in rats and NHPs demonstrated a substantially improved therapeutic index compared to the first generation LNP.

Clinical development

The translation of LNP-enabled siRNA systems for the treatment of hATTR amyloidosis in humans proceeded in two stages. The first generation DLinDMA-based formulation was evaluated in a placebo-controlled phase 1 trial to determine safety and efficacy of ALN-TTR01 after administration of a single dose, ranging from 0.01 to 1 mg kg^{-1}. The study provided a number of key insights: (1) NHP studies seemed to provide a reasonable prediction of human efficacy (approximately 50% mean TTR reduction observed in NHPs at 1 mg kg^{-1}); (2) ALN-TTR01 showed an encouraging safety profile with no drug-related serious adverse events or discontinuations or mild-to-moderate infusion-related reactions in a subset of participants; and (3) a single dose of ALN-TTR01 at 1 mg kg^{-1} led to a mean reduction in serum TTR levels of 38% compared to the placebo (Fig. 3a), with one patient achieving a substantial TTR reduction of >80%. These results validated the RNAi approach, the siRNA and the LNP platform in a real patient setting for the first time. Based on these results, the MC3-based 2nd generation

LNP (ALN-TTR02, patisiran) was advanced to the clinic in a first-in-human phase 1 trial[13]. As predicted by the preclinical studies, patisiran showed improved clinical activity compared to ALN-TTR01 with rapid, robust and durable suppression of TTR levels of >80%, compared to the placebo at doses between 0.15 and 0.5 mg kg^{-1} (Fig. 3b).

The encouraging efficacy and safety profile observed in the phase 1 study paved the way for further clinical development, culminating in the randomized, double-blinded, placebo-controlled phase 3 APOLLO study[3] and finally, in the regulatory approval of Onpattro in the US and EU, with the potential for approval in other jurisdictions.

Future prospects

The clinical development pathway followed by Onpattro paves the way for the clinical translation of LNP nanomedicines containing nucleic acid-based drugs to enable many novel therapeutics based on silencing or expressing target genes. The ethanol-dilution rapid mixing manufacturing process employing ionizable cationic lipids can be readily extended to encapsulate much larger negatively charged molecules such as mRNA[14,15] and effective transfection has been achieved in a variety of tissues in addition to the liver[16]. LNP systems containing mRNA show promise to target and use the liver as a bioreactor for the production of therapeutic proteins, such as monoclonal antibodies[17] and hormones[18] following i.v. administration. Alternatively, when administered by intradermal or intramuscular routes, LNP mRNA systems provide highly effective vaccines for infectious diseases such as the Zika virus[19] or influenza virus[20]. Finally, LNPs containing mRNA coding for programmable nucleases show considerable potential for gene editing in vivo[21,22]. Challenges remain, including achieving improved site-specific transfection as well as improving our ability to transfect extrahepatic tissues. However, the rapid advances of recent years suggest that it is just a matter of time before these challenges are overcome. ❐

Akin Akinc[1], Martin A. Maier[1], Muthiah Manoharan[1], Kevin Fitzgerald[1], Muthusamy Jayaraman[1], Scott Barros[1], Steven Ansell[2], Xinyao Du[2], Michael J. Hope[2], Thomas D. Madden[2], Barbara L. Mui[2], Sean C. Semple[2], Ying K. Tam[2], Marco Ciufolini[3], Dominik Witzigmann[3], Jayesh A. Kulkarni[3], Roy van der Meel[3] and Pieter R. Cullis[3,4*]

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Competing interests

A.A., M.A.M., M.M., K.F., M.J. and S.B. are employees of Alnylam Pharmaceuticals. S.A., X.D., M.J.H., T.D.M., B.L.M., S.C.S. and Y.K.T. are employees of Acuitas. P.R.C. has financial holdings in Acuitas.

Exhibit E

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

210922Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review
Office Director
Cross Discipline Team Leader Review
Clinical Review
Non-Clinical Review
Statistical Review
Clinical Pharmacology Review

Cross-Discipline Team Leader Review

Date	August 10, 2018
From	Nick Kozauer, MD
Subject	Cross-Discipline Team Leader Review
NDA#	210922
Applicant	Alnylam Pharmaceuticals, Inc.
Date of Submission	December 11, 2017
PDUFA Goal Date	August 11, 2018
Proprietary Name / Non-Proprietary Name	Onpattro™ (patisiran)
Dosage form(s) / Strength(s)	0.3 mg/kg administered intravenously every 3 weeks as a lipid complex injection (patients weighing 100 kg or more should receive doses of 30 mg)
Applicant Proposed Indication(s)/Population(s)	Treatment of adults with hereditary transthyretin-mediated amyloidosis (hATTR)
Recommendation on Regulatory Action	Approval
Recommended Indication(s)/Population(s)	Treatment of the polyneuropathy of hereditary transthyretin-mediated amyloidosis in adults

1. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

Hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) is an autosomal dominant disorder caused by mutations in the transthyretin (TTR) gene, located on chromosome 18q. Wild-type TTR protein is primarily synthesized in the liver and exists in a tetrameric state transporting thyroxine (T_4) and vitamin A (retinol) in association with retinol binding protein (RBP). More than 120 different point mutations in the TTR gene have been identified that lead to hATTR amyloidosis. A replacement of valine by methionine at position 30 (V30M) is the most common mutation causing hATTR amyloidosis. These mutations result in protein misfolding, aggregation, and deposition in the peripheral and central nervous system, heart, kidneys, eyes, bone, and gastrointestinal tract. Symptom onset typically occurs between 20 and 70 years of age, with death occurring within 5 to 12 years of onset, most commonly from cardiac dysfunction, infection, or cachexia. Many patients experience a prominent neuropathy defined by the presence of peripheral neuropathy and autonomic dysfunction [referred to as hATTR-polyneuropathy (hATTR-PN)]. The global prevalence of hATTR-PN is estimated to be between 5,000 and 10,000 persons, with the highest rates occurring in certain endemic countries such as Portugal and Sweden.

There are no FDA-approved treatments for hATTR amyloidosis.

Patisiran is a small interfering ribonucleic acid (siRNA) that directs sequence-specific degradation of TTR messenger RNA (mRNA) in the liver. The result is a reduction in both wild-type and mutant TTR protein. The dose of patisiran proposed for marketing has been demonstrated to reduce TTR levels by an average of 78%.

The applicant has provided data from Study ALN-TTR02-004 (Study 004, in this memorandum); an 18-month, randomized, double-blind, placebo-controlled trial in adult patients with hATTR-PN. This trial compared a 0.3 mg/kg dose of patisiran administered intravenously (IV) as a lipid nanoparticle (LNP) infusion every 3 weeks to placebo. An objective evaluation of polyneuropathy (consisting of a clinical neurological examination and tests of nerve conduction, sensation, and postural blood pressure) showed worsening at the expected rate in the placebo-treated patients through the 18 months of the trial, whereas the average scores in patisiran-treated patients showed a modest degree of numerical improvement. Approximately 57% of patisiran-treated patients demonstrated numerical improvements of polyneuropathy scores during the trial, compared to only approximately 7% of placebo-treated patients. The clinical meaningfulness of these results was confirmed by a similar pattern of findings on a patient-reported subjective assessment of the clinical impact of polyneuropathy. This pattern of stability and even improvement in the signs and symptoms of patients' polyneuropathy is unexpected in the natural history of the disease. The longer-term benefits of treatment are unknown, and the trial was not designed to, and did not, demonstrate an effect of treatment on survival. The efficacy results from Study 004 establish the effectiveness of patisiran for the treatment of polyneuropathy in adult patients with hATTR amyloidosis.

Study 004 did not enroll a population of patients with significant cardiac disease at baseline (patients could not have New York Heart Association function class 3 or 4 heart failure) [redacted] (b) (4) The trial evaluated the effects of patisiran on several exploratory cardiac biomarkers [echocardiographic parameters, N-terminal pro b-type Natriuretic Peptide (NT-proBNP), troponin-I], [redacted] (b) (4)

The risks associated with treatment with patisiran are acceptable, particularly given the strength of the efficacy results. Infusion-related reactions (IRRs) are

a known concern with LNP drugs, and occurred in 19% of patisiran-treated patients in Study 004, compared to 9% of placebo-treated patients. IRRs were considered serious in less than 1% of cases and most commonly involved flushing, back pain, nausea, abdominal pain, dyspnea, and headache. These IRRs only infrequently led to treatment interruption (5%) or discontinuation (less than 1%) and can be partly mitigated with a premedication regimen that will be recommended in labeling. Wild-type TTR reduction leads to reductions in vitamin A levels. Patients in the patisiran development program were instructed to supplement with the recommended daily allowance of vitamin A, and no vitamin A-related ocular toxicities were observed. Vitamin A supplementation will be recommended in labeling.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) is an autosomal dominant disorder caused by mutations in the transthyretin (TTR) gene. Wild-type TTR protein is primarily synthesized in the liver and exists in a tetrameric state transporting thyroxine (T_4) and vitamin A (retinol) in association with retinol binding protein (RBP). More than 120 different point mutations in the TTR gene have been identified that lead to hATTR amyloidosis. A replacement of valine by methionine at position 30 (V30M) is the most common mutation causing hATTR amyloidosis. These mutations result in protein misfolding, aggregation, and deposition in the peripheral and central nervous system, heart, kidneys, eyes, bone, and gastrointestinal tract. Symptom onset typically occurs between 20 and 70 years of age, with death occurring within 5 to 12 years of onset, most commonly because of cardiac dysfunction, infection, or cachexia. Many patients experience a prominent neuropathy defined by the presence of peripheral neuropathy and autonomic dysfunction [referred to as hATTR-polyneuropathy (hATTR-PN)]. • The global prevalence of hATTR-PN is estimated to be between 5,000 and 10,000 persons, with the highest rates occurring in certain endemic countries such as Portugal and Sweden.	HATTR amyloidosis is a serious disease, leading to significant disability and death.
Current Treatment Options	• There are no FDA-approved treatments for hATTR amyloidosis. Treatment in clinical practice is mainly supportive. Published literature suggests that orthotopic liver transplantation may be effective at stabilizing disease progression in some patients (e.g., patients with V30M mutations).	There are no FDA-approved treatments for hATTR amyloidosis.
Benefit	• Patisiran is a small interfering ribonucleic acid (siRNA) that directs sequence-specific degradation of TTR messenger RNA (mRNA) in the liver. The result is a reduction in both the wild-type and mutant TTR protein. The dose of patisiran proposed for marketing has been demonstrated to reduce TTR levels by an average of 78%.	This application has established that patisiran is effective for the treatment of polyneuropathy in adult patients with hATTR amyloidosis. Patisiran-treated patients in Study 004 demonstrated mean improvements from baseline on both objective and

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• The applicant has provided data from Study 004; an 18-month, randomized, double-blind, placebo-controlled trial in adult patients with hATTR-PN. This trial evaluated a 0.3 mg/kg dose of patisiran administered intravenously (IV) as a lipid nanoparticle (LNP) infusion every 3 weeks compared to placebo. The trial's primary efficacy analysis demonstrated a highly statistically significant treatment effect on the modified Neuropathy Impairment Scale +7 (mNIS+7); an objective evaluation of the signs and symptoms of polyneuropathy. Mean mNIS+7 scores in patisiran-treated patients demonstrated a modest numerical improvement during the trial, compared to the decline in the placebo-treated patients, which was expected. Approximately 57% of patisiran-treated patients demonstrated numerical improvements of polyneuropathy scores, compared to approximately 7% of placebo-treated patients. • A similar pattern of treatment effects was observed on the Norfolk Quality of Life Diabetic Neuropathy (Norfolk QoL-DN) scale, which supports the clinical meaningfulness of the objective mNIS+7 scores. • Study 004 also evaluated the effects of patisiran on several exploratory cardiac biomarkers [e.g., echocardiographic parameters, N-terminal pro B-type natriuretic peptide (NT-proBNP), and troponin-I] in a subgroup of patients (b) (4) [redacted]	subjective evaluations of polyneuropathy. This pattern of progression is very rare in the absence of treatment. The longer-term benefits of patisiran treatment are unknown. Study 004 was not designed to, and did not, demonstrate a benefit on survival. (b) (4) [redacted]
Risk and Risk Management	• Infusion-related reactions (IRRs) are a known concern with LNP drugs and were observed in the patisiran development program. Such reactions most commonly involving flushing, back pain, nausea, abdominal pain, dyspnea, and headache. They were considered serious in < 1% of cases and only infrequently led to treatment interruption (5%) or discontinuation (< 1%). IRRs can be partially mitigated with a premedication regimen (i.e., a corticosteroid, acetaminophen, and histamine blockers) that will be recommended in labeling. • Wild-type TTR reduction leads to reductions in vitamin A levels. Patients in the patisiran development program were instructed to supplement with the recommended daily allowance of vitamin A, and no vitamin A-related	The risks associated with treatment with patisiran are acceptable, particularly given the strength of the efficacy results. Risk management can be achieved through clear product labeling and routine postmarketing surveillance. The applicant will be required to establish a postmarketing pregnancy registry as there are no clinical data regarding the risk of treatment during pregnancy.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	ocular toxicities were observed. Vitamin A supplementation will also be recommended in labeling. • In the placebo-controlled study, 4 of 148 patients in the patisiran group (2.7%) versus 0 of 77 patients in the placebo group had serious adverse events of atrioventricular block requiring pacemaker support. Atrioventricular block could be a chance finding, but there are data suggesting that patisiran causes remodeling of the intraventricular septum, which could predispose to atrioventricular conduction defects. Although the risk is uncertain, complete heart block will be mentioned in Section 6 of labeling. • [redacted] (b) (4) In the placebo-controlled Study 004, 7 deaths in the patisiran group (4.7%) were possibly related to heart failure (cause characterized as sudden cardiac death or heart failure), whereas there was only one such death in the placebo group (with 2:1 randomization). Given that the numbers of event are small and causality is uncertain, these findings will not be included in labeling at this time. • There were no clinically significant differences between patisiran- and placebo-treated patients on hematologic parameters, thyroid parameters, coagulation parameters, liver function tests, electrolytes, or vital signs (outside the context of IRRs). Notably, patisiran-treated patients in Study 004 gained an average of 3 pounds during the 18-month trial, compared to an average 7-pound weight loss on placebo (a known manifestation of disease-related gastrointestinal dysautonomia).	The WARNINGS AND PRECAUTIONS section of the product labeling will describe the risk of infusion-related reactions and potential risk of vitamin A deficiency and the need for supplementation. Complete heart block is clinically important; however, causality is uncertain. Balancing these factors, complete heart block will be mentioned in the ADVERSE REACTIONS section of labeling.

2. Background

This application contains data in support of the safety and effectiveness of patisiran, administered as a lipid nanoparticle (LNP) formulation for intravenous (IV) infusion, for the treatment of hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis). Patisiran is a new molecular entity (NME) that has not been approved for any indication and has not previously been the subject of any marketing application. There are no FDA-approved treatments for hATTR amyloidosis.

HATTR amyloidosis is a life-threatening autosomal dominant disorder, caused by more than 120 identified mutations in the transthyretin (TTR) gene, located on chromosome 18q. Wild-type TTR protein (also referred to as prealbumin) is primarily synthesized in the liver (and to a lesser extent in the choroid plexus and retinal pigment epithelium) and exists in a tetrameric state, transporting thyroxine (T4) and vitamin A (retinol) in association with retinol-binding protein (RBP). The various hATTR amyloidosis mutations lead to misfolding of the TTR protein, which results in protein aggregation and amyloid deposition in the peripheral and central nervous system, heart, kidneys, eyes, bone, and gastrointestinal tract. A replacement of valine by methionine at position 30 (V30M) is the most common mutation causing hATTR amyloidosis. Symptom onset typically occurs between 20 and 70 years of age. Death generally occurs within 5 to 12 years after onset, most often because of cardiac dysfunction, infection, or cachexia.

Three forms of the disease are often described, although patients will often experience more than one form clinically. The neuropathic form [hereditary transthyretin-mediated amyloidosis polyneuropathy (hATTR-PN), historically referred to as transthyretin familial amyloid polyneuropathy (TTR-FAP)], is defined by the presence of peripheral neuropathy and autonomic dysfunction. The leptomeningeal form is defined by the presence of stroke, intracranial hemorrhage, hydrocephalus, ataxia, spastic paralysis, seizures, dementia, psychosis, and vision impairment. The cardiac form [hereditary transthyretin-mediated amyloidosis cardiomyopathy (hATTR-CM)] is defined by the presence of arrhythmia, cardiomegaly, heart failure, and death.

The global prevalence of hATTR-PN is estimated to be between 5,000 and 10,000 individuals, with the highest rates occurring in certain endemic countries such as Portugal and Sweden.

Patisiran is a small interfering ribonucleic acid (siRNA) molecule targeting TTR messenger RNA (mRNA) in the liver. RNA interference (RNAi) is a process by which siRNA, typically 21-23 nucleotides in length, directs sequence-specific degradation of target mRNA. When synthetic siRNAs are introduced into cells, they bind to complementary mRNA sequences, which results in the cleavage of the target mRNA and the suppression of its product protein. Patisiran suppresses the production of both wild-type and mutant TTR protein.

The applicant has provided data from a single adequate and well-controlled clinical trial as the primary basis of support of the effectiveness of patisiran for the treatment of hATTR-PN. The trial enrolled a population of patients with hATTR-PN, and was designed to evaluate the efficacy of patisiran for the treatment of polyneuropathy. (b) (4) [redacted]

The regulatory history of the patisiran development program is detailed in Dr. Rainer Paine's clinical review.

3. Product Quality

The technical lead on the Office of Product Quality (OPQ) review was Dr. Martha Heimann (Dr. Heimann's review lists the entire OPQ team involved with the review of this application). OPQ recommends approval of this application. The OPQ review concludes that a (b) (4) [redacted]-month retest date should be granted for the drug substance when stored at (b) (4) [redacted]C in the proposed commercial container closure system, and that a 24-month expiration dating period should be granted for the drug product when stored refrigerated in the commercial packaging. All manufacturing facilities were found to be acceptable.

The OPQ review outlines a postmarketing commitment (PMC) regarding the development and validation of a new *in vitro* drug release method and the setting of the drug release acceptance criteria for the finished drug product. The review also discusses several post-approval quality agreements that have been reached between the applicant and OPQ during the review period. Comments for the action letter are provided related to these items.

4. Nonclinical Pharmacology/Toxicology

The nonclinical reviewer for this application was Dr. David Carbone, with Dr. Lois Freed performing a secondary review. Drs. Carbone and Freed conclude that the application is approvable from a pharmacology/toxicology standpoint. The following are among the principal conclusions from Dr. Carbone's review:

- There are no nonclinical safety concerns regarding excipients, impurities, or degradation products.

- Reversible effects in monkeys included reductions in circulating retinol binding protein, vitamin A, and thyroxine. These effects were expected based on the known interactions of these molecules with TTR.

- Transient increases in heart rate were observed in safety pharmacology studies conducted in monkeys with no apparent drug effects on central nervous system or respiratory function.

- The primary toxicity observed in both rats and monkeys was an elevation in liver enzymes, with hepatocyte vacuolation. Single-cell necrosis, reactive sinusoids, mixed-cell infiltration, and pigment deposition were observed in monkeys. Drug-related toxicities generally resolved during the recovery periods in both species. The No Observed Adverse Effect Level (NOAEL) in both species was 0.3 mg/kg, which was below the dose proposed for marketing when corrected for interspecies differences.

- There was no drug-related genetic toxicity or effect on reproduction or development. The 2-year carcinogenicity study in rodents was waived because of the expected reductions in drug exposure due to the formation of anti-drug antibodies (ADAs) in the 26-week pivotal

toxicology study. Carcinogenicity was therefore evaluated in a 26-week study in TgRasH2 mice. This study was adequately conducted and demonstrated that patisiran was not tumorigenic.

5. Clinical Pharmacology

An integrated Office of Clinical Pharmacology (OCP) review was written by Drs. Venkateswaran Chithambarampillai (the primary reviewer), Venkatesh Atul Bhattaram, Hobart Rogers, Theingi Thway, Christian Grimstein, Kevin Krudys, and Sreedharan Sabarinath (the clinical pharmacology team lead). The final signatory for the OCP review was Dr. Mehul Mehta. OCP recommends the approval of this application.

Table 1 summarizes the conclusions of the OCP review with respect to the pharmacologic and clinical pharmacokinetic properties of patisiran:

Table 1: Summary of OCP Review Findings

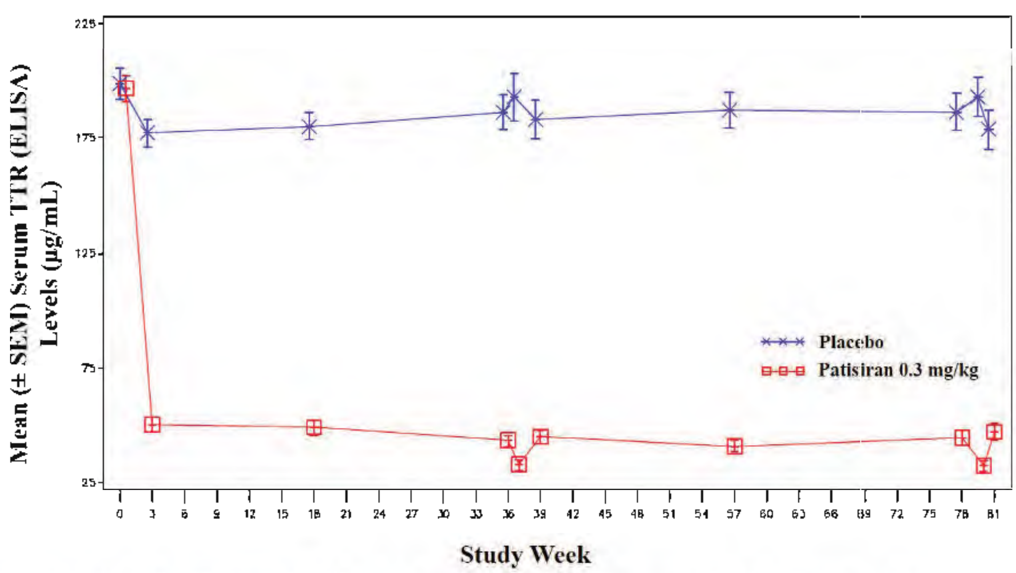
Mechanism of action	Patisiran siRNA binds to the RNA-Induced Silencing Complex (RISC) in the cytoplasm of human hepatocytes, which results in cleavage of double-stranded siRNA to a single strand within the RISC complex. Following hybridization of the single stranded siRNA-RISC complex with target TTR mRNA, the RISC complex uses the endonuclease argonaute 2 to cleave the TTR mRNA and thus inhibits the translation of mRNA into TTR protein. As previously noted, this mechanism of action suppresses both mutant and wild-type mRNA.
Pharmacokinetics	Following IV infusion, patisiran exposures [maximum plasma concentration (C_{max}) and area under the plasma concentration-time curve (AUC)] increase in a linear and dose-proportional manner.
Variability	Inter-individual variability in plasma C_{max} of patisiran (%CV) ranges from 30-38% and $AUC_{0\text{-}last}$ (%CV) for patisiran ranges from 84% to 110%.
Immunogenicity	Anti-drug antibodies (ADAs) specific to PEG2000-C-DMG were detected using a validated ELISA. Dr. Susan Kirshner, from the Office of Biotechnology Products (OBP), has determined that appropriate validation studies were performed to establish the suitability of the immunogenicity assay and concluded that the assay is suitable for its intended purpose (noting that assay sensitivity may range from 250 – 1000 ng/mL). In the placebo-controlled studies, ADAs were detectable in 4.1% of patisiran-treated patients (6/148). However, the ADA titers were low, and their appearance was transient. ADA status did not seem to influence the clinical efficacy, pharmacokinetic, or pharmacodynamic profiles of patisiran. However, the number of patients with ADA positive tests was too small to rule out ADA effects definitively.
Distribution	Patisiran is primarily distributed in the liver. Plasma protein binding is low (< 2.1%).
Metabolism	Patisiran is primarily metabolized by nucleases to shorter nucleotides of varying lengths.
Excretion	The mean terminal elimination half-life of patisiran is approximately 3 days. Less

	than 1% of administered patisiran is excreted unchanged in the urine.
QT prolongation	A Thorough QT (TQT) study was waived because of the low likelihood of direct ion channel interactions. Additionally, there is no evidence from nonclinical or clinical data that suggests that patisiran has the potential to delay ventricular depolarization.

In the pivotal effectiveness trial (Study 004), patisiran was dosed as a 0.3 mg/kg IV infusion administered over 80 minutes once every 3 weeks. Patients weighing 100 kg or more had their doses capped at 30 mg.

The following applicant figure, copied from the OCP review, demonstrates that patients receiving patisiran in Study 004 achieved an average 78% reduction in serum TTR protein levels, compared to a 6% reduction in patients receiving placebo (assessments were obtained prior to dosing at the respective study visits). Maximum reductions in serum TTR protein levels were observed 7-10 days after the first dose, with a consistent effect through 18 months.

Figure 1: Average (± SEM) Reduction of Serum TTR Protein Levels over Time Between Patisiran- and Placebo-Treated Patients in Study 004



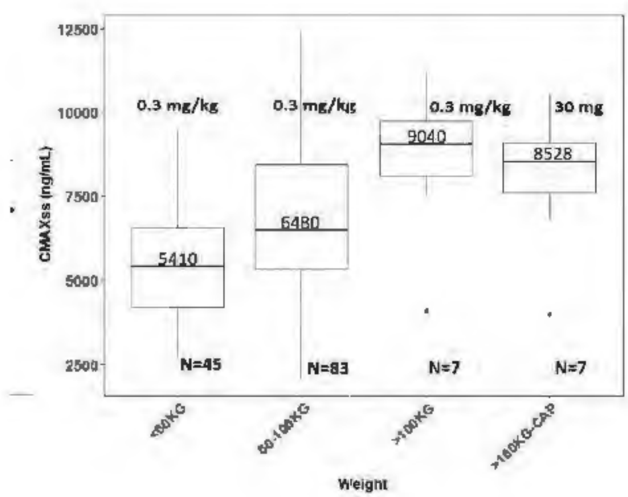
Source: Study ALN-TTR02-004; Module 5.3.5.1; section 14 Tables, Figures and Graphs; Figure 14.2.3.1

The OCP review notes that the applicant evaluated the effect of an every-4-week dosing regimen of patisiran on serum TTR protein reduction in a Phase 2 study. Such dosing resulted in only 63% suppression of serum TTR protein levels, which led the applicant to select an every-3-week regimen for Study 004.

The OCP review finds that intrinsic factors including age, race, gender, and renal or hepatic impairment do not impact the systemic exposures of patisiran, noting that such effects would not be anticipated as patisiran is primarily metabolized by nucleases. The OCP review indicates that there was a trend towards increasing exposures with increasing body weight. However, at the 0.3 mg/kg dose, exposures in patients weighing 100 kg or more were comparable to exposures normalized to a 30 mg equivalent dose. Therefore, the OCP review finds acceptable the applicant's proposal to cap

doses at 30 mg for patients weighing 100 kg or more. The following figure, copied from the OCP review, depicts the effect of body weight on patisiran exposures.

Figure 2: Effect of Body Weight on Patisiran Exposures



The OCP review notes that because patisiran is administered intravenously, a food-drug interaction is not expected. Additionally, *in vitro* drug interaction studies suggest that patisiran is not a substrate of or inhibitor for any major cytochrome (CYP) enzymes and transporters; therefore, the drug-drug interaction liability of patisiran is minimal.

Based on the preceding conclusions, the OCP review finds the applicant's proposed dosing regimen acceptable.

6. Clinical Microbiology

Not applicable.

7. Clinical/Statistical-Efficacy

Dr. Rainer Paine was the clinical reviewer for this application. Dr. Xiang Ling was the biometrics reviewer, and Dr. Kun Jin was the biometrics team lead.

Study 004 (APOLLO)

A single clinical trial, Study 004 (APOLLO), was intended to provide substantial evidence of the effectiveness of patisiran for the treatment of hATTR-PN.

Study 004 was an 18-month, randomized, double-blind, placebo-controlled trial in adult patients with polyneuropathy caused by hATTR. The trial was conducted at 44 investigational sites located in 19 countries. A total of 225 patients were randomized in a 2:1 ratio to receive 0.3 mg/kg doses of patisiran (patients weighing 100 kg or more received doses of 30 mg) administered intravenously every 3 weeks (n=148) or placebo (n=77). Randomization was stratified by baseline Neuropathy

Impairment Score (NIS) (<50 versus ≥50), early onset V30M mutations (<50 years of age at onset) versus all other mutations (including V30M with an age of onset ≥ 50), and the previous use of agents purported to stabilize TTR-protein tetramers (tafamidis or diflunisal) versus no previous use (neither drug is FDA-approved for the treatment of hATTR amyloidosis; tafamidis is approved in Europe and diflunisal is used off-label in many countries).

Patients were required to be between 18 to 85 years of age (inclusive) and have a diagnosis of hATTR amyloidosis with a documented mutation in the TTR gene. Patients were also required to have a baseline NIS score of 5 to 130 (higher scores indicate more severe polyneuropathy), a baseline polyneuropathy disability (PND) score of less than 3b (i.e., ambulatory with at most the help of one stick or crutch), and a Karnofsky performance status of greater than 60% (i.e., scores of 60% and lower indicate an increasing need for a reliance on others for self-care). Baseline nerve conduction studies (NCS) were also required to be supportive of the presence of polyneuropathy (the specific NCS enrollment criteria are defined in Dr. Paine's review). Patients with current use of purported tetramer stabilizers were excluded.

The primary efficacy endpoint was the change from baseline to Month 18 in modified NIS+7 (mNIS+7) scores. As Dr. Paine details in his review, the mNIS+7 is comprised of the NIS and the +7. The NIS is a clinical exam-based neuropathy evaluation [assessing both weakness (NIS-W) and reflexes (NIS-R)]; the +7 is an objective evaluation of small and large nerve fiber function [including NCS and quantitative sensory testing (QST)], as well as measurements of autonomic function (postural blood pressure). Scores range from 0 to 304, with higher scores indicating more severe neuropathy. There is a loose correlation between mNIS+7 scores and clinical function; however, the majority of patients with scores of 100 or greater will be reliant on the use of assist devices for ambulation.

Dr. Paine notes that the mNIS+7 is capable of detecting small changes in its components that are not obviously clinically meaningful. For that reason, it is important that the results of the analysis of the mNIS+7 be considered together with the results of the Norfolk-Quality of Life-Diabetic Neuropathy Scale (Norfolk-QoL-DN), which was the trial's first hierarchically-ordered secondary efficacy endpoint. The Norfolk-QoL-DN is a 35-item patient-reported measure that evaluates patients' perception of impairment with respect to physical functioning/large fiber neuropathy, activities of daily living, neuropathy symptoms, small fiber neuropathy, and autonomic dysfunction. The version of the Norfolk QoL-DN that was used in the trial had a maximum possible score of 136, with higher scores indicating greater impairment.

The primary analysis of the mNIS+7 was performed using a restricted maximum likelihood (REML)-based mixed-effects model repeated measures (MMRM) approach. The analyses of the Norfolk-QoL-DN and the other hierarchically-ordered secondary efficacy endpoints (described below) were performed using a similar model. Dr. Ling's review provides additional details of these analyses.

Study 004 enrolled 225 patients. The mean age was 61 years (range: 24 to 83 years). Three-quarters of patients were male, 72% were White/Caucasian, and 23% were Asian. Patients were from North America (21%), Western Europe (44%), and rest of world (36%). The mean disease duration since diagnosis was 2.5 years, and the majority (72%) of patients were more than 50 years of age at symptom onset. The mean baseline NIS was 59 points. Overall, 10% of patients had early-onset V30M mutations, and 53% of patients had previously used either tafamidis and/or diflunisal. Drs. Ling and Paine conclude that the baseline demographics and disease-specific characteristics were generally similar between the treatment arms.

Overall, 40 patients (18%) discontinued study treatment early (7% versus 38% in the patisiran- and placebo-treated groups, respectively), of whom 32 (14%) withdrew from the trial (7% versus 29% in the patisiran- and placebo-treated groups, respectively). The most common reasons for patient withdrawal were consent withdrawal, adverse events, and death.

The results of the primary efficacy analysis were highly statistically significant in favor of patisiran. At Month 18, mNIS+7 scores improved from baseline by least squares (LS) mean of 6 points in the patisiran group, whereas the LS mean score worsened by a mean of 28 points in the placebo group [LS mean difference: 34 points (*p*<0.001)]. Dr. Ling's review describes the results of a number of sensitivity analyses that support the finding of the primary analysis. The following table, reproduced from the application, present the results of the analysis of the mNIS+7.

Table 2: Study 004 – mNIS+7 Change from Baseline at Month 18 (mITT Population)

	Placebo (N=77)	**Patisiran (N=148)**
N contributing data	67	141
Baseline value (mean ± SD)	74.6 (37.0)	81.0 (41.5)
Δ from Baseline (LS Mean ± SEM)	28.0 (2.6)	-6.0 (1.7)
95% CI	22.8, 33.1	-9.5, -2.6
LS Mean (SEM) Difference (Patisiran - Placebo)	-	-34.0 (3.0)
95% CI	-	-39.9, -28.1
p-value	-	<0.001

SD: standard deviation; SEM: standard error of the mean; CI: confidence interval
The mNIS+7 score range is 0-304, with higher scores indicating more severe impairment.
The MMRM model includes baseline mNIS+7 as covariate and fixed-effect terms including treatment group, visit, treatment-by-visit interaction, genotype, age at hATTR symptom onset, previous tetramer stabilizer use, and region.

The results of the analysis of the Norfolk-QoL-DN were also highly statistically significant in favor of patisiran. At Month 18, Norfolk-QoL-DN scores improved compared to baseline (LS mean: -7 points), while placebo-treated patients worsened by a LS mean of +14 points [LS mean difference: -21 points (*p*<0.001)]. The following table, with format modified from the application, presents the results of the analysis of the Norfolk QoL-DN.

Table 3: Study 004 – Norfolk QoL-DN Change from Baseline at Month 18 (mITT Population)

	Placebo (N=77)	Patisiran (N=148)
N contributing data	65	141
Baseline value (mean ±SD)	55.5 (24.3)	59.6 (28.2)
Δ from Baseline (LS Mean ± SEM)	14.4 (2.73)	-6.7 (1.77)
95% CI	9.0, 19.8	-10.2, -3.3
LS Mean (SEM) Difference (Patisiran - Placebo)	-	-21.1 (3.10)
95% CI	-	-27.2, -15.0
p-value	-	<0.001

The Norfolk QoL-DN ranges from -4 to 136, with higher scores representing greater impairment.
MMRM model includes baseline Norfolk QoL-DN score as covariate and fixed-effect terms including treatment group, visit, treatment-by-visit interaction, baseline NIS, genotype, age at hATTR symptom onset, previous tetramer stabilizer use, and region.

The trial was not designed to demonstrate a statistical improvement in mNIS+7 or Norfolk QoL-DN scores from baseline. However, the fact that the patisiran-treated group numerically improved from baseline to Month 18 on both endpoints is remarkably inconsistent with the natural history of the disease over an 18-month duration.

The trial also evaluated 5 additional hierarchically-ordered polyneuropathy-focused secondary endpoints including the NIS-W (the weakness component of the NIS), the Rasch-built Overall Disability Scale (R-ODS), the 10-meter walk test (10MWT), modified-body mass index (mBMI), and the COMPASS-31 (an assessment of autonomic symptoms). The following table, modified from Dr. Ling's review, presents the results of these secondary endpoint analyses, which were all highly statistically significant in favor of patisiran.

Table 4: Study 004 – Results of Additional Hierarchically-Ordered Secondary Efficacy Endpoints

Endpoint	Change from Baseline at Month 18 LS Mean (SEM)		Patisiran - Placebo Treatment Difference LS Mean (95% CI)	*p*-value
	Patisiran	Placebo		
NIS-W[a]	0.05 (1.3)	17.9 (2.0)	-17.9 (-22.3, -13.4)	<0.001
R-ODS[b]	0.0 (0.6)	-8.9 (0.9)	9.0 (7.0, 10.9)	<0.001
10-MWT (m/sec)[b]	0.08 (0.02)	-0.24 (0.04)	0.31 (0.23, 0.39)	<0.001
mBMI[b]	-3.7 (9.6)	-119 (14.5)	116 (82, 149)	<0.001
COMPASS 31[a]	-5.3 (1.3)	2.2 (1.9)	-7.5 (-11.9, -3.2)	<0.001

[a] A lower number indicates less impairment/fewer symptoms.
[b] A higher number indicates better condition.

Dr. Ling also concludes that the efficacy of patisiran was consistent across subgroups defined by age, gender, race, and region.

Open-Label Extension Data

Dr. Paine's review discusses the findings from two uncontrolled open-label trials that were included in the application: Study ALN-TTR02-003 (N=27; complete), and Study ALN-TTR02-006 (N=64 at a 52-week interim analysis; ongoing). The results of the analyses of the various efficacy endpoints from these trials are presented in Dr. Paine's review. Dr. Paine notes that these data are challenging to interpret because of the lack of a concurrent control group and the potential for observer-bias in some of the outcome measures. However, Dr. Paine reasonably concludes that the apparent stability of the various clinical efficacy measures over the course of these trials is consistent with the clinical benefit observed in the double-blind phase of Study 004. Dr. Paine views these data as supportive of the clinical efficacy of patisiran in that context.

Exploratory Cardiac Endpoint Analyses

(b) (4)

[redacted] The study defined a cardiac subpopulation as patients with baseline left ventricular (LV) wall thickness of 1.3 cm or greater (as assessed by echocardiography) in the absence of a history of aortic stenosis or hypertension. Importantly, however, patients with New York Heart Association Functional Class of 3 or 4 heart failure were excluded from the study, a critically important patient subgroup. Other higher-risk patients were also excluded, including patients with type I diabetes, type II diabetes > 5 years' duration, acute coronary syndrome within the past 3 months, uncontrolled arrhythmia, and unstable angina.

Study 004 evaluated the change from Baseline to Month 18 in the following exploratory cardiac endpoints in the cardiac subpopulation: LV wall thickness, LV mass, LV ejection fraction (LVEF), LV longitudinal strain, N-terminal pro b-type natriuretic peptide (NT-proBNP), and troponin I. These analyses were also performed in the mITT population. Analyses of these endpoints were not controlled for multiple comparisons. Dr. Preston Dunnmon provided a consultative review from the Division of Cardiovascular and Renal Products, and details can be found in his review.

The cardiac subpopulation included 90 patisiran-treated patients and 36 placebo-treated patients, or roughly 55% of all patients in Study 004.

With respect to echocardiographic measures of LV morphology, LV wall thickness was the most direct measure assessed, thus having better precision than the other parameters with less propagation of error. According to Table 37 in the applicant's clinical study report, baseline mean (± SD) LV wall thickness was 16.4 ± 2.1 mm (n=36) in the placebo group, and 16.8 ± 2.6 mm (n=90) in the patisiran group. At Month 18, mean LV wall thickness was 16.2 ± 2.6 mm in the placebo group (n=25), and 15.4 ± 2.7 mm in the patisiran group (n=79). The least-squares mean changes (SEM) in mean LV wall thickness (baseline to Month 18, based on a Mixed-Effect Model Repeated Measure model) were -1.0 mm in the patisiran group and -0.1 mm in the placebo group (least-squares mean difference between groups: -0.9 mm, 95% CI: -1.69, -0.17).

The applicant also found that patisiran was associated with favorable effects in NT-proBNP. (Elevated NT-proBNP is associated with heart failure, worsening heart failure, and other states.) In the cardiac subpopulation, baseline mean NT-proBNP levels were 1512 ± 1754 (n=88) and 1318 ± 1469 ng/L

(n=34) in the patisiran and placebo groups, respectively. At Month 18, mean NT-proBNP was 1322 ± 1974 ng/L in the patisiran group (n=80) and 2943 ± 5748 ng/L in the placebo group (n=24). After database lock, the applicant observed that the NT-proBNP distributions were highly skewed, violating the MMRM model's assumption of normality. They applied a logarithmic transformation to normalize the distributions, and were able to conclude that patisiran had a nominally statistically significant treatment effect on NT-proBNP.

(b) (4)

[redacted]

(b) (4)

[redacted]

Efficacy Conclusions

The efficacy results from Study 004 support the approval of patisiran for the treatment of polyneuropathy in adult patients with hATTR amyloidosis. The study results have a number of characteristics, cited by FDA's 1998 Guidance "*Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products*," that support reliance on a single study. Patisiran's treatment effect

was highly statistically significant on the study's primary efficacy endpoint, and the results were robust to any reasonable sensitivity analysis. The study was large (given the size of the patient population) and multicenter; no single study site provided an unusually large fraction of the patients, and no single investigative site was disproportionately responsible for the observed treatment effect. The primary finding was supported by multiple secondary endpoints, all with rigorous control of the type-I error rate; and many of the secondary endpoints demonstrated salutary, yet distinct, treatment effects, confirming that the observed treatment differences were highly clinically meaningful. Results were generalizable across important subsets based on demographic and baseline disease characteristics. Finally, and importantly, the pharmacodynamic/mechanistic data, demonstrating a remarkable reduction in serum TTR, are consistent with the efficacy data.

The open-label extension data provided in the application are consistent with the efficacy results of Study 004 and provide some additional support of efficacy, though they were not needed, based on the strength and consistency of the placebo-controlled results.

Patisiran will be indicated for the treatment of polyneuropathy in adult patients with hATTR amyloidosis, (b) (4) [redacted]

8. Safety

Dr. Paine conducted the safety review of this application. Dr. Wiley Chambers from the Office of New Drugs (OND) and Dr. Kimberly Smith from DCRP conducted consultative safety reviews.

Dr. Paine's review indicates that a total of 240 subjects were exposed to patisiran in this development program, including 148 patients with hATTR amyloidosis who were exposed in Study 004. A total of 186 patients received patisiran for 12 months or more, 137 patients received patisiran for 24 months or more, and 52 patients received patisiran for 36 months or more. The large majority of these subjects were exposed to the dose of patisiran intended for marketing. All agree that the current safety database is adequate in the context of a rare disease such as hATTR amyloidosis.

In reviewing the applicant's adverse event data, translation of verbatim terms to preferred terms was inadequate in some cases, most commonly when one adverse event caused a second adverse event. For example, the verbatim term "left ankle and foot swelling secondary to fall" was given the preferred term "peripheral swelling," but the fall itself was not recorded as a preferred term for that evert. The verbatim term "fall secondary to worsening orthostatic hypotension" was translated to "fall," but "orthostatic hypotension" went unnoted. Having corrected these omissions, however, there were essentially no effects on the overall results of the adverse event analyses.

The following are the principal conclusions of Dr. Paine's safety review of the application:

There were 21 deaths in the patisiran development program, including 15 in patisiran-treated patients and 6 in placebo-treated patients. Approximately 60% of the deaths occurred in the randomized, placebo-controlled study (004), with 7 deaths in 148 patisiran-treated patients (4.7%) and 6 deaths in 77 placebo-treated patients (7.8%).

Originally, all 7 deaths in the patisiran group (4.7%) were considered to be cardiovascular in nature, with only 1 cardiovascular death in placebo-treated patients (1.3%). The applicant subsequently convened an independent and blinded adjudication committee to review the cases of death from Study 004 and classify them as cardiovascular or non-cardiovascular. Table 6 summarizes the results of this reclassification. With adjudication, all 7 deaths in the patisiran group remained attributed to cardiovascular causes, whereas the causes of death for 2 patients in the placebo group were re-classified from non-cardiovascular to cardiovascular (yellow highlighted cells), increasing the total number of cardiovascular deaths to 3 in the placebo group (3.9%). Importantly, however, both of the cardiovascular deaths added to the placebo group were attributed to stroke, not heart failure.

Table 6: External Independent & Blinded Adjudication Committee Classified Deaths as Cardiovascular (CV), non-CV, or Unknown.

Subject	Fatal Serious Adverse Event	Committee Classification	Days Since Last Dose	Study Day	Notes
Patisiran					
66 yo male	Cardiac arrest, CHF exacerbation	CV (heart failure)	191	194	History CHF, atrial fibrillation. Developed worsening CHF. Autopsy: Death due to complications of systemic TTR amyloidosis with extensive cardiac involvement.
66 yo male	Sudden cardiac death	CV (presumed sudden death)	64	169	History CHF, atrial fibrillation. Developed osteomyelitis, acute kidney injury, CHF exacerbation, UTI, CVA, acute heart failure
67 yo male	Sudden cardiac death	CV (sudden death)	378	381	History cardiomyopathy, CHF, atrial fibrillation. Developed shortness of breath on while climbing stairs and collapsed. Sudden death with amyloidosis, restrictive cardiomyopathy,malnutrition and anemia contributory.
42 yo female	CHF, acute pulmonary edema	CV (sudden death)	356	378	History cardiomyopathy, cardiac failure. Cause of death listed as acute pulmonary edema secondary to heart failure and amyloidosis.
64 yo male	Cardiac arrest	CV (presumed CV)	547	565	History NYHA class 2 CHF. Atrial fibrillation/flutter. During prolonged hospitalization for infected decubital ulcers, patient had cardiac arrest.
59 yo male	Pulseless electrical activity	CV (sudden death)	169	172	Hx: cardiomyopathy, palpitations, left bundle branch block. Prolonged episode of chest pain and palpitations at home, developed difficulty breathing became pulseless.
65 yo female	cardiac failure	CV (heart failure)	526	529	History NYHA class 2 CHF. Atrial fibrillation, COPD. Worsening of cardiac insufficiency.
Placebo					
57 yo male	Subarachnoid hemorrhage	CV (fatal stroke, hemorrhagic)	547	558	History NYHA class 2 CHF. Ischemic stroke (2007). Had sudden fall/cardio-respiratory arrest. Head CT: subarachnoid hemorrhage.
61 yo male	Staphylococcal sepsis	non-CV	380	407	History NYHA class 2 CHF. Had cardiac arrest, complicated course. Multiple vegetations and erosions of cardiac valves c/w staphylococcal endocarditis.
58 yo male	Anemia, gastrointestinal hemorrhage	CV (heart failure)	338	422	Hx: restrictive cardiomyopathy, CHF, AF, pulmonary fibrosis, orthostatic hypotension, scleroderma, hypothyroidism. Several hospitalizations for CHF exacerbations during study. Recent worsening of cardiac symptoms. Developed melena with anemia. Received transfusion 1 PRBC. Based on overall poor health and prognosis, transferred to palliative care.
43 yo male	Acute kidney failure, urinary tract infection, bacteremia	unknown	274	298	NYHA Class 1 CHF. Developed UTI, acute renal failure (oliguria and anuria), and E. coli sepsis.
77 yo female	Colorectal cancer metastatic	non-CV	379	558	NYHA class I CHF; colectomy for Stage 3 colon cancer. Had Metastatic recurrence of colorectal cancer.
56 yo female	Ischemic stroke	CV (fatal stroke, ischemic)	108	134	History NYHA class 2 CHF. Atrial fibrillation/flutter. Had ischemic stroke, complicated by exacerbation of CHF and pneumonia.

Thus, with respect to deaths plausibly related to heart failure, the 7 to 1 difference (4.7% in the patisiran group vs. 1.3% in the placebo group) remains, and this difference is concerning. With only small numbers of cardiovascular deaths, the finding does not merit description in labeling. But these findings are not reassuring with respect to patients with heart failure, (b) (4) [redacted]

The incidence of serious adverse events (SAEs) was similar in the patisiran and placebo groups in Study 004, 37% and 40%, respectively. The incidence of adverse events that led to treatment discontinuation was higher in the placebo group (14.3%) than in the patisiran group (4.7%).

One serious adverse event of note was atrioventricular block: in the patisiran group, there were 3 subjects with complete atrioventricular block and a fourth subject with atrioventricular block. All 4 serious adverse events were deemed to be severe and all led to pacemaker insertion. These findings will be noted in the Adverse Reactions Section of labeling (Section 6). If it is true that patisiran causes intraventricular septal thinning in patients with cardiac involvement, it is plausible that there is remodeling and/or inflammation within the septum, in turn affecting atrioventricular conduction. Although the number of cases of serious atrioventricular block was low, they are nevertheless concerning, and would be of particular concern if patisiran were indicated for hATTR-cardiomyopathy.

Table 7, reproduced from the information in Dr. Paine's review, summarizes the most common treatment-emergent adverse events (TEAEs) that occurred in Study 004.

Table 7: Adverse Reactions from Study 004 that Occurred in at Least 5% of Patisiran-treated Patients and at Least 3% More Frequently than in Placebo-treated Patients

Preferred Term	Patisiran N=148 %	Placebo N=77 %
Peripheral edema	30	22
Upper respiratory tract infections[a]	29	21
Infusion-related reaction[b]	19	9
Dyspepsia	8	4
Muscle spasms	8	1
Arthralgia [c]	7	0
Dyspnea [c]	7	0
Erythema [c]	7	3
Bronchitis	7	3
Vertigo	5	1

[a] Includes nasopharyngitis, upper respiratory tract infection, respiratory tract infection, pharyngitis, rhinitis, sinusitis, viral upper respiratory tract infection, upper respiratory tract congestion.
[b] Infusion-related reaction symptoms include, but are not limited to: arthralgia or pain (including back, neck, or musculoskeletal pain), flushing (including erythema of face or skin warm), nausea, abdominal pain, dyspnea or cough, chest discomfort or chest pain, headache, rash, chills, dizziness, fatigue, increased heart rate or palpitations, hypotension, hypertension, facial edema.

[c] Not part of an infusion-related reaction

As indicated in the table, 19% of patisiran-treated patients experienced an IRR in Study 004, compared to 9% of placebo-treated patients. IRRs led to infusion interruption in 5% of patients, and to permanent treatment discontinuation in less than 1%. The most common symptoms (reported in greater than 2% of patients) of IRRs were flushing, back pain, nausea, abdominal pain, dyspnea, and headache. There was only one reported serious adverse event (hypotension and syncope) related to an IRR; it occurred in the expanded access program.

Because of the risk of IRRs, a premedication regimen consisting of a corticosteroid (typically dexamethasone), acetaminophen, and antihistamines (H1 and H2 blockers) was used during the development program. Dr. Paine's review contains a detailed discussion of this issue, including the recommended agents and doses. Initially, a premedication regimen was used both on the night prior to study treatment, and on the day of study treatment. However, the regimen was reduced following the occurrence of corticosteroid-related serious adverse events. The reduced premedication regimen eliminated dosing on the night prior to treatment and reduced doses of both the corticosteroid and the H2 blocker on the day of study treatment. There was no increase in the incidence of IRRs with the reduced premedication regimen.

There were no clinically significant differences between patisiran- and placebo-treated patients on hematologic parameters, thyroid parameters, or coagulation parameters. There were also no clinically significant effects of patisiran on vital signs (outside of the context of IRRs), with the exception of mean body weight, which numerically increased by 3 pounds in patisiran-treated

patients at Month 18 in Study 004 and decreased by 7 pounds in placebo-treated patients (weight loss is common in the disease, because of gastrointestinal dysautonomia).

In Study 004, mean and median increases from baseline in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were greater in the patisiran group than in the placebo group. However, except for two confounded outlier cases discussed in Dr. Paine's review, ALT and AST elevations were all less than three times the upper limit of normal (ULN) (with the clear majority being less than two times the ULN). These elevations were sporadic and resolved despite continued treatment, with no clinically significant sequelae. There were no Hy's Law cases.

A higher percentage of patisiran-treated patients had a shift from a normal baseline potassium level to at least one high post-baseline value in Study 004, compared to placebo-treated patients (24% versus 8%). Similarly, a higher percentage of patisiran-treated patients had a shift from normal baseline sodium levels to at least one low post-baseline value in Study 004, compared to placebo-treated patients (3.4% versus 1.3%). These changes were sporadic and transient with no clinically significant sequalae.

There was no effect of patisiran on mean or median blood urea nitrogen (BUN), creatinine, or estimated glomerular filtration rate (eGFR). Five patisiran-treated patients did have significant but transient increases in BUN and creatinine, with decreased eGFRs. These abnormalities resolved despite continued treatment and there were no other significant outliers in renal function. There were no nonclinical signals of renal toxicity with patisiran. Dr. Kimberly Smith from the Division of Cardiovascular and Renal Products provided a focused renal consultation regarding these 5 cases. Dr. Smith notes that the pattern of renal abnormalities observed in these cases was unusual and concluded that they are largely unexplained based on the information provided by the applicant. In any case, however, the transient nature of these events, the lack of any other apparent signals of renal toxicity, and the lack of any nonclinical renal safety signals suggest a low-likelihood that these findings are treatment-related.

Although extravasation was observed in less than 0.5% of infusions, infusion site extravasation led to infusion interruption in 2% of patients. Two serious adverse events associated with extravasation were reported in Study 004 including post-infusion cellulitis/superficial thrombophlebitis and dermatitis.

Wild-type TTR transports vitamin A in association with RBP. Because patisiran reduces both wild-type and mutant TTR, patients in the clinical development program were instructed to take the recommended daily allowance of vitamin A. The applicant also evaluated the ocular safety of patisiran in the development program. Dr. Wiley Chambers, the ophthalmology consultant, conducted a review of the ocular safety data. Dr. Chambers found no evidence of ocular vitamin A deficiency with patisiran and agreed that the applicant's suggestion to supplement patients with vitamin A in clinical practice is appropriate. The product label will recommend that patients be referred to an ophthalmologist if they develop ocular symptoms suggestive of vitamin A deficiency (e.g., night blindness).

Overall, the risks associated with patisiran are acceptable to support approval. There will be warnings/precautions for IRR and the need for vitamin A supplementation. Atrioventricular block will be mentioned in the Adverse Reactions section of labeling. Given the small number of cases, this conduction abnormality does not rise to the level of a Warning/Precaution.

9. Advisory Committee Meeting

This application was not referred for review to an advisory committee because the safety profile of patisiran is acceptable for the intended population, the clinical trial design is acceptable, and the efficacy findings were clear.

10. Pediatrics

Pediatric Research Equity Act (PREA) requirements were not triggered for this orphan indication.

11. Other Relevant Regulatory Issues

No Good Clinical Practice (GCP) issues were identified in Dr. Paine's review.

Dr. Paine concludes that the applicant has adequately disclosed financial interests/arrangements with clinical investigators.

The Office of Scientific Investigations (OSI) investigated two clinical investigator sites, the applicant, and the contract research organization (CRO) (Medpace). The inspection results from the two clinical investigator sites were classified as No Action Indicated (NAI). The OSI review notes that there were some significant deviations from Good Clinical Practice (GCP) at the applicant and CRO inspections, but these findings are unlikely to significantly impact data reliability.

Patisiran is currently under review by the European Medicines Agency (EMA) as a marketing authorization application. The OSI review also notes that EMA shared its inspection findings from clinical investigations of a site in Mexico and Spain, respectively. The EMA found that a number of deficiencies that were identified at the Spanish site rendered the data suboptimal, and recommended that these data be excluded from their analyses. However, a sensitivity analysis conducted by Dr. Ling that excluded this site did not significantly impact the primary efficacy results.

The Controlled Substance Staff (CSS) reviewer for this application was Dr. Edward Hawkins. Dr. Hawkins noted that at the October 19, 2017, pre-NDA meeting, the applicant was informed that there was no need to submit an abuse potential assessment with the application. Dr. Hawkins reviewed the current application and continued to conclude that there is no abuse potential with patisiran.

12. Labeling

Please refer to the final negotiated product label. Labeling negotiations with the applicant have been completed and the applicant has accepted all recommended changes.

13. Postmarketing Commitments/Requirements

The Division of Risk Management (DRISK) reviewer for the application was Dr. Yasmeen Abou-Sayed. Dr. Abou-Sayed concluded that a risk evaluation and mitigation strategy (REMS) is not necessary for patisiran.

The following will be a postmarketing commitment:

- The development and validation of a new *in vitro* drug release method and the setting of the drug release acceptance criteria for the finished drug product.

The following will be a postmarketing requirement:

- Establish a worldwide Pregnancy Surveillance Program to collect and analyze information for a minimum of 10 years on pregnancy complications and birth outcomes in women exposed to patisiran during pregnancy.

14. Recommended Comments to the Applicant

The action letter will include comments that reflect several post-approval quality agreements that have been reached between the applicant and OPQ during the review period.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

NICHOLAS A KOZAUER
08/10/2018

WILLIAM H Dunn
08/10/2018

ELLIS F UNGER
08/10/2018
I was involved in drafting this memorandum and agree with its contents.

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: August 6, 2018
From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 210-922 (Onpattro, Patisiran-LNP, ALN-TTR02)

NDA 210-922 was submitted by the sponsor (Alnylam Pharmaceuticals) to support approval of ALN-TTR02 for the treatment of adults with hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) with polyneuropathy. The NDA was a rolling application, with the CMC and nonclinical portions provided on November 15, 2017, and the remainder on December 11, 2017. The sponsor was notified that the NDA was filed for Priority review, with no potential review issues identified, on February 1, 2018. Clinical development of ALN-TTR02 was conducted under IND 117395.

To support clinical development and the NDA, the sponsor conducted an acceptable battery of nonclinical studies of ALN-TTR02 (patisiran-LNP), including following:

- Pharmacology (in vitro and in vivo)
- Safety pharmacology (in vitro hERG; in vivo CNS, cardiovascular, respiratory parameters in monkey)
- PK/ADME/TK
- Toxicology
    - Sprague-Dawley rat (4-, 6-, and 26-week IV infusion; 13/19-week SC)
    - Cynomolgus monkey (6- and 39-week IV infusion)
- Reproductive and developmental toxicology (IV infusion)
    - Fertility and early embryonic development in male Sprague-Dawley rat
    - Combined fertility and early embryonic development and embryofetal development in female Sprague-Dawley rat
    - Embryofetal development in New Zealand White rabbit
    - Pre- and postnatal development in Sprague-Dawley rat
- Carcinogenicity (IV bolus)
    - 26-week carcinogenicity in Tg.rasH2 mouse
- Genetic toxicology
    - Ames assay
    - In vitro chromosomal aberration assay in HPBL
    - In vivo micronucleus assay in CD-1 mouse (IV bolus)

Additional studies (including in vitro genetic toxicology assays) were conducted to further qualify two novel excipients in the LNP (or lipid complex) formulation, DLin-MC3-DMA and PEG_{2000}-C-DMG.

The nonclinical data were reviewed by Dr. Carbone (Review and Evaluation of Pharmacology/Toxicology, David Carbone, Ph.D., NDA 210-922, July 11, 2018). Dr. Carbone conducted a thorough review of the nonclinical data and has concluded that they support approval of the NDA, with no post-marketing requirement recommendations. The following provides only a brief summary of selected findings in the nonclinical studies; full details and discussion are provided in Dr. Carbone's review.

Pharmacology

Primary: Patisiran is a double-stranded siRNA designed to reduce levels of transthyretin (TTR) protein through RNAi-mediated degradation of wild type and mutant TTR mRNA. The drug product, ALN-TTR02 (patisiran-LNP), is a patisiran-containing lipid nanoparticle (or lipid complex) for targeted delivery of drug to the liver. Patisiran is active in human and nonhuman primate but not in rodent. It appears that only one in vivo PD study (20031783) was conducted with ALN-TTR02. (Other PD studies were conducted using different lipid nanoparticle formulations.) In that study, ALN-TTR02 was administered to cynomolgus monkeys (2/sex/group) at IV doses of 0.15-0.50 mg/kg Q4W or 0.25-0.30 mg/kg Q3W to males and females (2/sex/group). Reductions in serum TTR protein were observed in all groups, with steady-state achieved after 3-4 doses. Greater suppression was observed at higher doses when given at the same frequency; greater frequency (at the same dose) resulted in similar maximum, but more prolonged, suppression. Similar maximum and minimum reductions of serum TTR protein (>95% and >80%, respectively) were obtained with 0.3 mg/kg Q3W and 0.5 mg/kg Q4W.

Secondary: No dedicated secondary pharmacology study was conducted with ALN-TTR02. However, TTR is known to be involved in the transport of retinol, by stabilizing retinol binding protein and preventing its renal elimination, and thyroxine. To assess for potential drug-related effects, circulating levels of Vitamin A and thyroxine were assessed in the toxicity studies.

Safety Pharmacology: Effects of ALN-TTR02 on CNS, cardiovascular, and respiratory parameters were assessed in cynomolgus monkey. ALN-TTR02 was administered as a single 1-hr IV infusion at doses of 0.1, 1, 3, and 6 mg/kg. There were no drug-related effects on CNS or respiratory parameters, except for an increase (1-3 °C) in body temperature at >1 mg/kg. The only cardiovascular finding was a prolonged (39-47 hrs post dose) increase in heart rate at >1 mg/kg, which was considered possibly related to the increased body temperature.

PK/ADME

The PK/ADME of ALN-TTR02 was assessed in Sprague-Dawley rat and cynomolgus monkey. In rat, plasma, spleen, and liver concentrations of patisiran, DLin-MC3-DMA, and PEG_{2000}-C-DMG were quantitated following acute IV bolus doses of 0.03, 0.3, and 1 mg/kg of patisiran; corresponding doses of DLin-MC3-DMA were 0.20, 2.03, and 6.76 mg/kg, respectively, and of

PEG_{2000}-C-DMG were 0.02, 0.23, and 0.76 mg/kg, respectively. Plasma PK parameters are summarized in the following table (patisiran doses in mg/kg; units: C_{max} (ng/mL), AUC (ng*hr/mL), $t_{1/2}$ (hr), CL (mL/hr/kg), V_{SS} (mL/kg); TTR02-NCD10-006).

PARAMETER	**MALES**			**FEMALES**		
	0.03	**0.3**	**1**	**0.03**	**0.3**	**1**
PATISIRAN						
C_{max}	587	7070	26650	595	7730	25100
$AUC_{(0-\infty)}$	298	3402	--*	308	--	13825
$t_{1/2}$	0.28	0.29	--	0.23	--	0.44
CL	101	88.2	--	97.3	--	72.3
V_{SS}	39.8	27.5	--	32.8	--	31.2
DLin-MC3-DMA						
C_{max}	3715	40850	150500	3550	43150	140500
$AUC_{(0-\infty)}$	6007	67875	228266	6257	70505	220741
$t_{1/2}$	82.3	200	246	177	184	288
CL	33.3	29.9	29.6	32.0	28.8	30.6
V_{SS}	2125	3849	3797	4035	4761	5966
PEG_{2000}-C-DMG						
C_{max}	[redacted]	[redacted]	16500	[redacted]	[redacted]	15100
$AUC_{(0-\infty)}$	[redacted]	[redacted]	--	[redacted]	[redacted]	20807
$t_{1/2}$	[redacted]	[redacted]	--	[redacted]	[redacted]	21.3
CL	[redacted]	[redacted]	--	[redacted]	[redacted]	36.5
V_{SS}	[redacted]	[redacted]	--	[redacted]	[redacted]	304

*not reportable

Liver and spleen PK parameters are provided in the following table (sexes combined; units: C_{max} (ng/g), AUC (ng*hr/g)).

PARAMETER	**LIVER**			**SPLEEN**		
	0.03	**0.3**	**1**	**0.03**	**0.3**	**1**
PATISIRAN						
C_{max}	NE	1529	5481	180	837	1051
$AUC_{(0-t)}$	NE	5991	30269	468	11098	15200
DLin-MC3-DMA						
C_{max}	3180	33725	128750	1993	8770	22400
$AUC_{(0-t)}$	698661	9715397	36691537	418564	3837639	12144920
PEG_{2000}-C-DMG						
C_{max}	[redacted]	[redacted]	7988	[redacted]	[redacted]	2123
$AUC_{(0-13)}$	[redacted]	[redacted]	201179	[redacted]	[redacted]	196480

In monkey, plasma and liver concentrations for patisiran, DLin-MC3-DMA, and PEG_{2000}-C-DMG were quantitated following acute 1-hr IV infusion doses of 0.03, 0.3, and 1 mg/kg of patisiran; corresponding doses of DLin-MC3-DMA were 0.20, 2.03, and 6.76 mg/kg, respectively, and of PEG_{2000}-C-DMG were 0.02, 0.23, and 0.76 mg/kg, respectively. Plasma PK parameters are summarized in the following table (patisiran doses in mg/kg; units: C_{max} (µg/mL), AUC (µg*hr/mL), $t_{1/2}$ (hr), CL (mL/hr/kg), V_{SS} (mL/kg); TTR02-NCD10-018).

PARAMETER	MALES			FEMALES		
	0.03	**0.3**	**1**	**0.03**	**0.3**	**1**
PATISIRAN						
C_{max}	0.258	3.50	16.5	0.203	3.92	18.1
$AUC_{(0-\infty)}$	0.402	6.68	20.7	0.754	--	36.6
$t_{1/2}$	3.52	8.44	14.7	10.2	--	19.1
CL	164	46.3	49.5	135	--	29.8
V_{SS}	278	447	328	409	--	264
DLin-MC3-DMA						
C_{max}	2.02	27.4	122	1.82	31.5	136
$AUC_{(0-\infty)}$	63.1	645	1860	50.7	727	1557
$t_{1/2}$	508	780	550	428	441	766
CL	3.19	3.24	3.83	4.04	2.92	4.49
V_{SS}	1310	1550	2090	1460	1510	2190
PEG_{2000}-C-DMG						
C_{max}			16.8			18.2
$AUC_{(0-\infty)}$			226			221
$t_{1/2}$			160			162
CL			3.48			3.49
V_{SS}			141			128

Liver PK parameters are summarized in the following table (patisiran doses in mg/kg; units: C_{max} (ng/g), AUC (ng*day/g); NE = not estimable).

PARAMETER	MALES			FEMALES		
	0.03	**0.3**	**1**	**0.03**	**0.3**	**1**
PATISIRAN						
C_{max}	NE	65.0	205	NE	88.0	162
$AUC_{(0-t)}$	--*	--	--	--	--	--
DLin-MC3-DMA						
C_{max}	6580	85100	264000	7420	82300	281000
$AUC_{(0-t)}$	102175	1244850	3684000	92707	1480600	4530900
PEG_{2000}-C-DMG						
C_{max}			1920			3400
$AUC_{(0-13)}$			5519			6189

*not reportable

Toxicology

Pivotal (GLP) IV toxicity studies of ALN-TTR02 were conducted in male and female Sprague-Dawley rat and cynomolgus monkey.

In rat, ALN-TTR02 (1-hr IV infusion) was assessed in 3 GLP toxicity studies. In the 6-week study (+60-day recovery), ALN-TTR02 was administered at doses of 0 (PBS), 0.15, 0.8, 1.8, and 3 mg/kg Q2W; a separate group received AF-011-1955 (non-pharmacologically active siRNA against insect luciferase, in same LNP formulation) at an IV dose of 3 mg/kg Q2W. No NOAEL was identified because of liver findings (including hepatocellular necrosis and reactive sinusoidal lining cells) at all doses of ALN-TTR02 and with AF-011-1955 (suggesting LNP-mediated toxicity). The only microscopic findings at the end of the recovery period were in male

reproductive organs (degeneration/atrophy of the seminiferous epithelium and oligo/aspermia in epididymis), which was observed at the end of the dosing period in one male in each of the higher dose groups (MD and HD).

The TK data are summarized in the following table (NC = not calculated; units: patisiran dose (mg/kg), C_{max} (ng/mL), AUC (ng*hr/mL; samples collected up to 337 and 1465 hrs after end of infusion on Day 1 and Day 43, respectively)).

SAMPLING DAY	PARAMETER	MALES				FEMALES			
		0.15	0.8	1.8	3	0.15	0.8	1.8	3
1	**PATISIRAN**								
	C_{max}	1025	5890	13050	24850	1050	7470	14200	25950
	$AUC_{(0-t)}$	1548	6870	21678	36973	1474	11608	23973	42083
	DLin-MC3-DMA								
	C_{max}	8310	40600	105500	152500	6655	46700	109000	199500
	$AUC_{(0-t)}$	20344	106299	210131	603712	19412	118259	279810	484100
	PEG_{2000}-C-DMG								
	C_{max}	921	4465	11250	23350	709	5010	11850	20500
	$AUC_{(0-t)}$	2279	13398	36122	77973	1917	14262	34534	61797
43	**PATISIRAN**								
	C_{max}	819	4505	18700	35400	1189	1652	15245	32850
	$AUC_{(0-t)}$	NC	4628	19341	38458	1218	2594	18188	35801
	DLin-MC3-DMA								
	C_{max}	4140	29450	110500	248000	7525	9970	93250	229000
	$AUC_{(0-t)}$	21891	122652	280887	623718	20782	77675	257619	695382
	PEG_{2000}-C-DMG								
	C_{max}	802	3595	12200	25950	870	2085	11585	24950
	$AUC_{(0-t)}$	2516	16980	35355	54376	2192	12320	35567	57515

Because an NOAEL was not established, a 4-week study was conducted at ALN-TTR02 doses of 0 (PBS), 0.1, 0.3, and 1.0 mg/kg Q4W. Microscopic findings in liver (including single cell or hepatocellular necrosis and reactive sinusoidal lining cells) were again observed at all doses. The sponsor identified the LD as an NOAEL because of the minimal severity of single cell necrosis and the lack of accompanying increases in LFTs (ALT, AST, alkaline phosphatase, and total bilirubin), which were observed at >0.1 mg/kg. (ALT and AST were increased at all doses in the 6-week study). The male reproductive organ effects observed in the 6-week Q2W study were not detected in the 4-wk Q4W study. The 4-week study is less relevant to clinical use because of the less frequent dosing, compared to that proposed for humans (Q3W).

In the 26-week (+12-week recovery) study, ALN-TTR02 was administered at doses of 0 (0.9% saline), 0.03, 0.1, and 0.3 mg/kg Q2W. There were 11 unscheduled deaths during the study, but none was considered drug-related. The primary findings were injection site reaction (vascular/perivascular inflammation), evident in all groups (slight increase in severity with dose), and microscopic changes in liver (periportal vacuolation) at the HD. Reversibility was demonstrated for both findings. This study was not an adequate assessment of the chronic effects of ALN-0TTR02 because of the lack of detectable patisiran in plasma at the Day 183 sampling time (samples were collected for TK only on Days 1 and 183). Antibodies to PEG_{2000}-C-DMG were detected in all dose groups and in 10 control animals; however, plasma levels did not

correlate with the incidence of anti-PEG antibodies in individual animals. The sponsor suggested that antibody development or injection site inflammation may have resulted in the decrease in plasma patisiran exposure.

The TK data are summarized in the following table (LLOQ = 25 ng/mL; ND = not determined or insufficient data; units: patisiran dose (mg/kg), C_{max} (ng/mL), AUC (ng*hr/mL)).

PARAMETER	**MALES**						**FEMALES**					
	0.03	**0.1**	**0.3**	**0.03**	**0.1**	**0.3**	**0.03**	**0.1**	**0.3**	**0.03**	**0.1**	**0.3**
	DAY 1			**DAY 183**			**DAY 1**			**DAY 183**		
PATISIRAN												
C_{max}	231	1250	3300	<LLOQ	<LLOQ	132	30.80	1040	1510	<LLOQ	667	<LLOQ
$AUC_{(0-\infty)}$	ND	2590	4380	ND	ND	ND	ND	1650	ND	ND	ND	ND
DLin-MC3-DMA												
C_{max}	812	8090	24600	40.5	36.3	1670	147	5000	11400	13.1	4600	277
$AUC_{(0-\infty)}$	3930	29300	89900	4830	13800	ND	1510	16400	45100	2340	15200	ND
PEG_{2000}-C-DMG												
C_{max}	107	1050	2880	20.4	37.3	544	38.7	890	1600	5.45	483	196
$AUC_{(0-\infty)}$	282	2850	8990	ND	1110	9620	ND	ND	4970	ND	ND	9650

In monkey, ALN-TTR02 (1-hr IV infusion) was assessed in 2 GLP toxicity studies; both were conducted in a minimal number of animals (3/sex/group). In the 6-week study (+60-day recovery), ALN-TTR02 was administered at doses of 0 (PBS), 0.3, 1.0, and 3 mg/kg Q2W. A separate group received AF-011-1955 (3 mg/kg Q2W). The primary findings were microscopic changes in liver (including centrilobular vacuolation, single cell necrosis, and reactive sinusoidal cells) at the HD of ALN-TTR02 and with AF-011-1955; these changes were accompanied by increases in ALT and AST. (Increases in ALT and AST were also observed at the lower doses but were not accompanied by liver histopathology.) Pharmacological activity (decreased serum and liver TTR) was observed at all doses in a dose-related manner (99% at HD), accompanied by decreases in serum Vitamin A (>80% at HD) and thyroxine (<50%); reversibility was demonstrated, except that Vitamin A levels were still reduced at the end of the recovery period at the HD. TK parameters are summarized in the following table (sexes combined; patisiran dose in mg/kg; units: C_{max} (µg/mL), AUC (µg*hr/mL; last sampling time was last measurable concentration)).

PARAMETER	**DAY 1**			**DAY 43**		
	0.3	**1**	**3**	**0.3**	**1**	**3**
PATISIRAN						
C_{max}	2.94	14.7	62.2	3.31	18.4	70.2
$AUC_{(0-t)}$	6.37	38.7	167	8.12	45.0	156
DLin-MC3-DMA						
C_{max}	26.5	120	534	24.5	132	379
$AUC_{(0-t)}$	348	998	4060	785	2420	6230
PEG_{2000}-C-DMG						
C_{max}	4.04	16.6	54.6	4.26	16.5	56.7
$AUC_{(0-t)}$	67.5	238	789	67.5	286	697

In the 39-week (+13-week recovery) study, ALN-TTR02 was initially administered at the same doses used in the 6-week study but Q3W instead of Q2W. However, after the unscheduled death of a HDF (found dead on Day 23; COD not determined) and increases in liver-associated clinical chemistry parameters (AST, ALT, alkaline phosphatase, GGT, bilirubin, and LDH) in HDM and HDF after the first dose, the HD was lowered to 2.0 mg/kg Q3W. (AF-011-1955 was not tested.) As in the 6-week study, the primary findings were microscopic changes (including centrilobular vacuolation, single cell necrosis, and reactive sinusoidal cells), at the MD and HD in males and in HDF, associated with increases in ALT, AST, and alkaline phosphatase at the HD. Pharmacological activity (decreased serum TTR) was demonstrated at all doses (97-99% at HD), accompanied by reduced serum Vitamin A (70-80%) and thyroxine (≤50%); reversibility of the findings was demonstrated. No drug-related ophthalmology or electroretinography findings were observed. TK parameters are summarized in the following table (patisiran dose in mg/kg; units: C_{max} (µg/mL), AUC (µg*hr/mL; last sampling time was 504 hrs after end of infusion on Day 1 and 2184 hrs after end of infusion on Day 274); ND = not determined).

PARAMETER	**MALES**						**FEMALES**					
	0.3	**1.0**	**3.0**	**0.3**	**1.0**	**2.0**	**0.3**	**1.0**	**3.0**	**0.3**	**1.0**	**2.0**
	DAY 1			**DAY 274**			**DAY 1**			**DAY 274**		
PATISIRAN												
C_{max}	3.54	10.4	43.1	4.63	12.3	35.8	3.15	10.0	50.6	4.52	14.3	34.5
$AUC_{(0-\infty)}$	13.1	26.0	134	23.9	57.9	89.5	11.2	23.5	218	ND	58.0	128
DLin-MC3-DMA												
C_{max}	33.5	86.3	265	34.9	112	330	30.0	87.2	277	41.2	129	318
$AUC_{(0-\infty)}$	683	3350	4750	2590	6040	8730	1030	2080	5290	1580	5520	7460
PEG_{2000}-C-DMG												
C_{max}	3.66	13.1	56.36	4.47	15.8	33.6	3.37	12.1	50.3	4.21	15.7	32.4
$AUC_{(0-\infty)}$	64.2	276	955	84.6	339	722	60.0	243	632	73.2	313	552

Reproductive and Developmental Toxicology

ALN-TTR02 (1-hour infusion) was tested in a full battery of reproductive and developmental toxicology studies, which consisted of a fertility study in male Sprague-Dawley rat, a combined fertility and embryofetal development study in female Sprague-Dawley rat, an embryofetal development study in New Zealand White rabbit, and a pre- and postnatal development study in Sprague-Dawley rat.

No adverse effects on fertility were observed in male rats administered ALN-TTR02 at IV doses of 0 (0.9% saline), 0.03, 0.1, or 0.3 mg/kg Q2W or the rodent (pharmacologically active) surrogate (AF-011-18534; 0.1 mg/kg Q2W). Reductions in serum TTR (~85%) and Vitamin A (~80%) were reduced only in the surrogate group. Serum thyroxine levels were reduced primarily in the surrogate group (~70%) but also slightly (15-20%, not dose-related) in ALN-TTR02 groups.

No adverse effects on fertility, embryofetal development, or pre- and postnatal development were observed in female rats administered ALN-TTR02 at IV doses of 0 (0.9% saline), 0.15, 0.5, and 1.5 mg/kg or AF-011-018534 (1.5 mg/kg) QW. In the pre- and postnatal development study, lipid components (DLin-MC3-DMA and PEG_{2000}-C-DMG) were detected in milk at all but the

LD; patisiran and the surrogate siRNA were not detected in milk at any dose. Pharmacological activity of the surrogate was evidenced by decreases in serum TTR, Vitamin A, and thyroxine in dams. In both studies, serum TTR (normalized to protein concentration) was decreased in all groups (~20-30%, including controls) but fell below the LLOQ in the surrogate group. Serum Vitamin A was decreased in all ALN-TTR02 groups (6-22%) only in the combined fertility and embryofetal development study; however, Vitamin A was reduced in the surrogate group in both studies (75-88%). Thyroxine was reduced in the surrogate group in both studies (20-66%). In the pre- and postnatal development study, serum TTR was slightly and transiently (~12% on PND 21) reduced in offspring of dams dosed with the surrogate; serum Vitamin A and thyroxine were not affected in offspring.

TK data for ALN-TTR02 in F_0 dams are summarized in the following table (patisiran dose in mg/kg; units: C_{max} (µg/mL), AUC (µg*hr/mL; "last" sampling time was ~72 hrs post infusion); GD 19 data are from the combined fertility and embryofetal development study; LD 18 data are from the pre- and postnatal development study.

PARAMETER	**PATISIRAN**			**Dlin-MC3-DMA**			**PEG$_{2000}$-C-DMG**		
	0.15	**0.5**	**1.5**	**0.15**	**0.5**	**1.5**	**0.15**	**0.5**	**1.5**
GESTATION DAY 19									
C_{max}	1.99	6.28	29.0	12.5	31.5	133	1.63	4.22	17.8
$AUC_{(0\text{-}24\ h)}$	4.62	16.0	88.0	30.1	99.1	361	3.98	11.6	41.1
LACTATION DAY 18									
C_{max}	0.49	1.20	3.14	4.02	2.84	2.82	0.62	1.35	2.82
$AUC_{(0\text{-}last)}$	1.02	1.50	6.18	19.10	27.90	72.50	3.42	5.36	15.10

According to the study report, TK parameters at the HD for patisiran and at all doses for the lipid components were affected by "individual concentrations that were not quantifiable due to being above the limit of quantitation with insufficient sample volume for reassay," which resulted in data from <3/sampling time. Whether this issue may have been responsible for the substantially lower exposures on LD 18 (in the pre- and postnatal development study) compared to GD 19 (in the embryofetal development study) is uncertain.

In rabbit, no adverse effects on embryofetal development were observed when ALN-TTR02 was administered at doses of 0 (0.9% saline), 0.1, 0.3, or 0.6 mg/kg IV QW; however, in a dose-ranging study (0 (0.9% saline), 0.3, 1, and 2 mg/kg IV QW), the MD and HD were associated with abortions, increased early resorptions, and post-implantation loss, and reduced fetal body weight and mean litter size, which were considered drug-related and associated with evidence of maternal toxicity (reduced body weight gain and food consumption).

Carcinogenicity

The carcinogenic potential of ALN-TTR02 was tested in a 26-week IV study in Tg.rasH2 mouse at doses of 0 (0.9% saline), 0.5, 2, and 6 mg/kg Q2W. There were no drug-related increases in tumors; the main non-neoplastic finding was single cell necrosis in liver (HDM).

The Division concluded that a 2-year carcinogenicity study in rat was not feasible, based on the substantial decrease in systemic exposure to patisiran, with no detectable levels near the end of the dosing period (Day 185), in the 26-week toxicity study.

Genetic Toxicology

ALN-TTR02 was negative in an adequately conducted standard battery of genotoxicity assays (Ames assay, in vitro chromosomal aberration in HPBLs, in vivo micronucleus assay in CD-1 mouse). The in vitro assays were conducted with and without metabolic activation (Aroclor 1254-induced rat liver S9).

Conclusions and Recommendations

A battery of nonclinical studies was conducted on ALN-TTR02. There are several issues regarding the nonclinical data, i.e., the lack of chronic toxicity and carcinogenicity data in rat and the lack of safety margins between exposures achieved in rodent and nonrodent studies and that expected in humans with the recommended clinical dosing regimen, even when exposures were normalized by dosing interval as proposed by the sponsor.

Pharmacokinetic data in humans following chronic administration of Onpattro at the recommended human dose of 0.3 mg/kg Q3W are summarized in the following table:

Patisiran		DLin-MC3-DMA		PEG_{2000}-C-DMG	
C_{max} (μgmL)	$AUC_{(0-t)}$ (μg*hr/mL)	C_{max} (μgmL)	$AUC_{(0-t)}$ (μg*hr/mL)	C_{max} (μgmL)	$AUC_{(0-t)}$ (μg*hr/mL)
7.15	184.05	42.5	1403	4.22	145.27

The lack of chronic toxicity and carcinogenicity data in rat were due to the development of anti-PEG antibodies, which resulted in plasma patisiran exposures below the LLOQ in the 26-week study and precluded the conduct of a meaningful 2-year carcinogenicity study. Although higher doses (as used in the reproductive and developmental toxicity studies) might have been tolerated in females in the 26-week study, it is unclear if that would have resulted in a longer duration of exposure. The lack of a safety margin in male rat and in monkey may be because of greater sensitivity to the toxicity of ALN-TTR02 in animals compared to humans.

The data indicate that liver was the primary target organ for toxicity in rodent (mouse, rat) and nonrodent (monkey), which appears to be due to the lipid components. Pharmacologically mediated effects consisted of decreases in TTR and associated decreases in serum Vitamin A and, to a lesser extent, thyroxine. In humans, Onpattro administration resulted in decreases in circulating TTR and Vitamin A but not thyroxine.

Although marked decreases in serum Vitamin A were observed in adult rats (administered the surrogate) and in monkeys, no adverse effects attributable to Vitamin A deficiency were observed. Ophthalmologic changes are considered a sensitive index of Vitamin A status; however, no adverse ocular effects were observed. Vitamin A is essential for reproduction and normal fetal development (Zile MH J Nutr 128:455S-458S, 1998; Maden M Postgrad Med J 77:489-491, 2001; Zile MH J Nutr 131:705-708, 2001; See AWM et al. Dev Biol 316:171-190,

2008; Gutierrez-Mazariegos J et al. Sem Cell Dev Biol 22:603-610, 2011); however, no adverse reproductive or developmental effects were observed and serum levels of Vitamin A in the offspring were unaffected by administration of the surrogate to dams throughout gestation and lactation. These data suggest that transport of Vitamin A to extra-hepatic tissues may not rely entirely on binding to the retinol binding protein (RBP): TTR complex or that serum Vitamin A levels in ALN-TTR02-dosed animals may not be a reliable marker of Vitamin A status (Paik J et al. J Nutr 134:276S-280S, 2004; Quadro L et al. Mole Aspect Med 24:421-430, 2003). Further discussion of this complex issue (Blaner WS et al. Subcell Biochem 81:95-125, 2016) is beyond the scope of this memo.

Overall, the nonclinical studies of ALN-TTR02 support approval of the NDA for the proposed indication. No post-marketing requirements are recommended.

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/s/

LOIS M FREED
08/06/2018

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 210922
Submission date: November 11, 2017 (nonclinical)
Drug: patisiran
Applicant: Alnylam Pharmaceuticals, Inc.
Indication: Treatment of adults with hereditary transthyretin-mediated amyloidosis

Reviewing Division: Division of Neurology Products

Discussion:
The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. Both found the information sufficient to support approval.

The carcinogenic potential of patisiran was assessed in a 6-month transgenic Tg.rasH2 mouse study. No drug-related tumors were noted. A 2-year carcinogenicity study in rats was not considered feasible because of the inability to maintain exposure due to anti-drug antibodies.

No nonclinical post-marketing requirements were recommended.

Conclusions: I agree that this NDA can be approved from a pharm/tox perspective.

Comments on labeling were provided separately.

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/s/

PAUL C BROWN
08/06/2018

CLINICAL REVIEW

Application Type	NME User Fee Program
Application Number(s)	NDA 210922
Priority or Standard	Priority
Submit Date(s)	December 11, 2017
PDUFA Goal Date	August 11, 2018
Division/Office	Division of Neurology Products/Office of Drug Evaluation 1
Reviewer Name(s)	Rainer W. Paine, MD, PhD
Review Completion Date	August 6, 2018
Established/Proper Name	Patisiran
(Proposed) Trade Name	Onpattro™
Applicant	Alnylam Pharmaceuticals, Inc.
Dosage Form(s)	Small Molecule (Drug)
Applicant Proposed Dosing Regimen(s)	0.3 mg/kg every 3 weeks as an IV infusion
Applicant Proposed Indication(s)/Population(s)	Treatment of adults with hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis)
Recommendation on Regulatory Action	Approval
Recommended Indication(s)/Population(s) (if applicable)	Treatment of the polyneuropathy of hereditary transthyretin amyloidosis in adults

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Glossary

AC	advisory committee
AE	adverse event
ALT	alanine transaminase
ANC	absolute neutrophil count
AR	adverse reaction
AST	aspartate transaminase
BLA	biologics license application
BPCA	Best Pharmaceuticals for Children Act
BRF	Benefit Risk Framework
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CMAP	Compound Muscle Action Potential
CMC	chemistry, manufacturing, and controls
COSTART	Coding Symbols for Thesaurus of Adverse Reaction Terms
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSR	clinical study report
CSS	Controlled Substance Staff
DMC	data monitoring committee
ECG	electrocardiogram
eCTD	electronic common technical document
ETASU	elements to assure safe use
FAP	Familial amyloidotic polyneuropathy, also known as hATTR amyloidosis with polyneuropathy
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	good clinical practice
GRMP	good review management practice
hATTR-CM	hereditary transthyretin amyloidosis with cardiomyopathy
hATTR-PN	hereditary transthyretin amyloidosis with polyneuropathy
ICF	Informed Consent Form
ICH	International Council for Harmonization
IENFD	intraepidermal nerve fiber density

IND	Investigational New Drug Application
INR	international normalized ratio
IRS	Interactive Response System
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
LNP	lipid nanoparticle
MAD	multiple ascending dose
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
MMRM	Mixed effect Model Repeated Measures
mNIS+7	Modified Neurologic Impairment Score +7
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Event
NCS	nerve conduction study
NCS Σ5	NCS sum of 5 attributes
NDA	new drug application
NME	new molecular entity
NIS	Neurologic Impairment Score
NIS+7	Neurologic Impairment Score +7
NIS LL	Neurologic Impairment Score Lower Limb
NIS-R	NIS reflexes
NIS-S	NIS sensation
NIS-W	NIS weakness
Norfolk QOL-DN	Norfolk Quality of Life-Diabetic Neuropathy
OCS	Office of Computational Science
OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PBRER	Periodic Benefit-Risk Evaluation Report
PD	pharmacodynamics
PI	prescribing information or package insert
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PND	Polyneuropathy Disability
PP	per protocol
PPI	patient package insert
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
PSUR	Periodic Safety Update report

QST-BSAHP	QST heat pain by body surface area
QST-BSA_TP	QST touch pressure by body surface area
HRdB	Heart rate variability with deep breathing
REMS	risk evaluation and mitigation strategy
RBP	Retinol Binding Protein
SAE	serious adverse event
SAP	statistical analysis plan
SGE	special government employee
SGNFD	sweat gland nerve fiber density
SNAP	sensory nerve action potential
SOC	standard of care
TEAE	treatment emergent adverse event
TTR-FAP	transthyretin familial amyloid polyneuropathy
TTR	transthyretin
ULN	upper limit of normal
VDT	Vibration detection threshold

1. Executive Summary

1.1. Product Introduction

In hereditary transthyretin amyloidosis (hATTR amyloidosis), misfolding of the transthyretin (TTR) protein leads to its aggregation and the formation of amyloid fibrils, which interfere with the normal function of affected organ systems, including the peripheral nervous system, which was evaluated for this product.

Patisiran (Onpattro™; called ALN-TTR02 in clinical trials) is a small interfering ribonucleic acid (siRNA) which targets TTR messenger ribonucleic acid (mRNA) in order to suppress the production of TTR.

Patisiran is a new molecular entity (NME) containing no previously approved active ingredients.

Note that reviewer commentary throughout the text is presented in *italics*.

1.2. Conclusions on the Substantial Evidence of Effectiveness

The placebo-controlled efficacy study ALN-TTR02-004 (Study 004) is a single adequate and well-controlled study that can support approval of patisiran for the treatment of adults with hereditary transthyretin amyloidosis with polyneuropathy (hATTR-PN), also known as transthyretin familial amyloid polyneuropathy (TTR-FAP).

Study 004 was a well-designed, multinational, 18-month study that has provided reliable, clinically meaningful, and statistically strong evidence of an effect of patisiran on polyneuropathy in patients with hATTR. The mean scores on the study's primary efficacy endpoint in patisiran-treated patients were notably somewhat improved from baseline, which is inconsistent with the known course of the condition. Although the trial was not designed to demonstrate that patients statistically improved on treatment, it is clear that treatment with patisiran resulted in at least the stability of polyneuropathy in many patients. The results of the primary analyses from this trial were also supported by highly statistically significant positive results on all of the trial's pre-specified secondary efficacy endpoints. Although more difficult to interpret because of their uncontrolled nature, the results of two open-label studies showing apparent clinical stability of subjects receiving patisiran further support the results of Study 004.

1.3. Benefit-Risk Assessment

Benefit-Risk Integrated Assessment

Patisiran (Onpattro™; called ALN-TTR02 in clinical trials) is a small interfering ribonucleic acid (siRNA) that targets transthyretin (TTR) messenger ribonucleic acid (mRNA) in order to suppress the production of TTR protein. Clinical studies evaluated patients with hereditary transthyretin amyloidosis with polyneuropathy (hATTR-PN). Based on the positive results of a single adequate and well-controlled study and confirmatory evidence from two open-label studies, it is the conclusion of this reviewer that the effectiveness of patisiran has been established for the treatment of adults with hATTR-PN.

Hereditary transthyretin amyloidosis (hATTR amyloidosis) is an autosomal dominant disorder (>120 TTR gene mutations known) that is characterized by the slowly progressive buildup of amyloid protein in the peripheral and central nervous systems, heart, kidneys, eyes, bone, and gastrointestinal tract. Death usually occurs within 5-12 years after onset, most often due to cardiac dysfunction, infection, or cachexia. The exact incidence of hATTR amyloidosis is unknown and varies geographically, but is estimated to be 1/100,000 in U.S. Caucasians. Approximately 100 to 2500 individuals are estimated to have hATTR-PN in the United States. These patients typically develop sensorimotor polyneuropathy with numbness, pain, and weakness, as well as focal nerve lesions (e.g., carpal tunnel syndrome) and autonomic dysfunction (e.g., orthostatic hypotension).

There is no approved drug for hATTR amyloidosis. Treatment options for hATTR amyloidosis include liver transplant and medical management of associated symptoms. Diflunisal, a non-steroidal anti-inflammatory drug, is sometimes used off-label to treat the disease. There is a significant unmet clinical need for effective treatments for hATTR amyloidosis.

An 18-month placebo-controlled multinational study of patisiran in adults with hATTR-PN (148 patisiran, 77 placebo control) evaluated polyneuropathy through clinical neurological examinations and tests of nerve conduction, sensation, and postural blood pressure. These test results worsened in the placebo-treated patients during the course of the trial, as would be expected from disease progression. In contrast, the average scores in the patisiran-treated patients improved modestly from baseline. Whereas only approximately 7% of placebo-treated patients improved, approximately 57% of patisiran-treated patients demonstrated numerical improvements of polyneuropathy scores during the trial. There was a similar pattern of positive results on a patient-reported subjective assessment of the clinical impact of polyneuropathy on their quality of life.

The most commonly observed (10% or greater) adverse reactions associated with the use of patisiran in the 18-month placebo-controlled study

were upper respiratory tract infection (29%) and infusion-related reactions (19%). The adverse reaction that caused the most patients to stop taking patisiran across all clinical studies was cardiac failure (2 patients in the placebo-controlled study, 1.4%). The proportion of patients experiencing serious adverse reactions was 37% in the patisiran group and 40% in the placebo group. Four (2.7%) of patisiran-treated patients experience a serious adverse reaction of heart block, including 3 cases of complete heart block. No serious adverse reactions of heart block were reported on placebo. There were more serious adverse events of diarrhea in the patisiran group (5.4% vs. 1.3% in placebo).

The conclusion of this review is that substantial evidence of clinical effectiveness and an acceptable safety profile have been established to support an approval of patisiran for the treatment of polyneuropathy in adults with hATTR.

Benefit-Risk Dimensions

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Hereditary transthyretin amyloidosis (hATTR amyloidosis) is a genetic disease that causes slowly progressive buildup of amyloid protein in the peripheral and central nervous systems, heart, kidneys, eyes, bone, and gastrointestinal tract. • Death usually occurs within 5-12 years after symptom onset, most often due to cardiac dysfunction, infection, or cachexia. • The incidence of hATTR amyloidosis is 1/100,000 in U.S. Caucasians.	HATTR amyloidosis is a serious disease that can lead to disability and death.
Current Treatment Options	• There is no approved drug for hATTR amyloidosis. • Treatment options for hATTR amyloidosis include liver transplant and medical management of associated symptoms. • Diflunisal, a non-steroidal anti-inflammatory drug, is sometimes used off-label to treat the disease.	There is a significant unmet clinical need for effective treatments for hATTR amyloidosis.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Benefit	• In the 18-month placebo-controlled multinational study of patisiran in adults with hATTR-PN (148 patisiran, 77 placebo control), there was an average treatment-effect of 34 points (baseline ~80; 304-point scale) on the Modified Neurological Impairment Score +7 (mNIS+7), an objective evaluation of a range of signs and symptoms related to polyneuropathy, for patients in the patisiran group compared to placebo ($p=9.3\times10^{-24}$). • There was an average treatment-effect of 21 points (baseline ~60; 141-point scale)on the Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) score, a patient-reported assessment of the impact of their polyneuropathy, for patients in the patisiran group compared to placebo ($p = 1.1 \times10^{-10}$) • There was statistically significant treatment benefit in the patisiran group compared to placebo for all secondary endpoints, including measures of disability (R-ODS), gait speed (10-MWT), nutritional status (mBMI), and autonomic symptoms (COMPASS 31). • Further confirmation of the benefit of patisiran in treating patients with hATTR-PN is provided by the results of two open-label studies. The results of these studies do not contradict the results of the main placebo-controlled study in adults with hATTR-PN described above.	The results of the 18-month placebo-controlled multinational study of patisiran in adults with hATTR-PN provide reliable, clinically meaningful, and statistically strong evidence that patisiran is effective for the treatment of polyneuropathy in the patients.
Risk and Risk Management	• The safety database for patisiran includes all patients from the Phase 3 controlled study and the open-label studies. • The most commonly observed (10% or greater) adverse events associated with the use of patisiran in the 18-month placebo-controlled study were upper respiratory tract infections and	The safety profile of patisiran is acceptable to support an approval. WARNINGS and PRECAUTIONS should be included in labeling to

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	infusion reactions. • The adverse event that caused the most patients to stop taking patisiran across all clinical studies was cardiac failure (2 patients in the placebo-controlled study, 1.4%). • In the 18-month placebo-controlled study, all-cause mortality was numerically lower in the patisiran group [7/148 (4.7%)] than in the placebo group [6/77 (7.8%)]. The 7 deaths in the patisiran group were related to cardiac causes (heart failure or arrhythmia), compared to 1/6 placebo deaths. However, the number of deaths was too small to make any conclusions about this finding. • Four (2.7%) of patisiran-treated patients experience a serious adverse reaction of heart block, including 3 cases of complete heart block. No serious adverse reactions of heart block were reported on placebo. • Infusion related reactions included flushing (6.0% across all studies), back pain (5.5%), nausea (3.7%), dyspnea (2.8%), and headache (2.3%). • Hypotension occurred in 1.4% of patisiran patients in the placebo-controlled study, compared to 0% in the placebo group. • One patient in the expanded access program experienced hypotension and syncope during the patisiran infusion. • In order to decrease the risk of infusion related reactions, all clinical study subjects received premedication regimens that included corticosteroids, H1/H2 blockers, and paracetamol or equivalents. Two patients had severe adverse events that were most likely related to corticosteroid use: osteomyelitis, osteopenia, and tibia	describe the risks of infusion-related reactions and the need for vitamin A supplementation to avoid possible vitamin A deficiency. DOSAGE AND ADMINISTRATION in labeling should include the premedication regimen used in the placebo-controlled study of patisiran to reduce the risk of infusion-related reactions.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	fracture. Additional adverse events that were possibly due to premedication included dizziness, insomnia, and somnolence. • There were two reports of serious adverse events in the placebo-controlled trial associated with extravasation of patisiran (post-infusion cellulitis/superficial thrombophlebitis, and dermatitis). • Patisiran reduces vitamin A levels in the body. All patients received the recommended daily amount of vitamin A as a supplement.	

1.4. Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

X	The patient experience data that was submitted as part of the application include:			Section where discussed, if applicable
	X	Clinical outcome assessment (COA) data, such as		
		X	Patient reported outcome (PRO)	6.1.1
		X	Observer reported outcome (ObsRO)	6.2.2
		X	Clinician reported outcome (ClinRO)	6.1.1
		X	Performance outcome (PerfO)	6.1.1
	□	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)		
	□	Patient-focused drug development or other stakeholder meeting summary reports		
	□	Observational survey studies designed to capture patient experience data		
	□	Natural history studies		
	□	Patient preference studies (e.g., submitted studies or scientific publications)		
	□	Other: (Please specify)		
□	Patient experience data that were not submitted in the application, but were considered in this review:			
		□	Input informed from participation in meetings with patient stakeholders	
		□	Patient-focused drug development or other stakeholder meeting summary reports	
		□	Observational survey studies designed to capture patient experience data	
		□	Other: (Please specify)	
□	Patient experience data was not submitted as part of this application.			

2. Therapeutic Context

- **Analysis of Condition**

Analysis of Condition

Hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) is an autosomal dominant disorder (>120 TTR gene mutations known) that is characterized by the slowly progressive buildup of amyloid protein in the peripheral and central nervous systems, heart, kidneys, eyes, bone, and gastrointestinal tract. TTR is a tetrameric protein primarily produced in hepatocytes. The disease is caused by genetic mutations in the TTR gene that lead the tetrameric TTR protein to break into monomeric units that misfold and aggregate as amyloid fibril deposits.

There are three general forms of the disease, although patients can have overlapping symptoms from all three forms. The neuropathic form (hereditary TTR amyloidosis polyneuropathy [hATTR-PN], also known as transthyretin familial amyloid polyneuropathy [TTR-FAP]), is defined by the presence of sensorimotor peripheral neuropathy (with symptoms of numbness, pain, and weakness), focal nerve lesions (e.g., carpal tunnel syndrome), autonomic dysfunction (e.g., orthostatic hypotension, gastrointestinal dysfunction), vitreous opacity of the eye, and glaucoma. The leptomeningeal form is defined by the presence of stroke, intracranial hemorrhage, hydrocephalus, ataxia, spastic paralysis, seizures, dementia, psychosis, and vision impairment. The cardiac form is defined by the presence of arrhythmia, cardiomegaly, heart failure, and death.

The exact incidence of hATTR is unknown and varies geographically, but is estimated to be 1/100,000 in U.S. Caucasians. Between approximately 100 to 2500 individuals are estimated to have hATTR-PN in the United States (Schmidt et al., 2018).

Symptom onset occurs between 20 and 70 years of age. Death usually occurs within 5-12 years after diagnosis, most often due to cardiac dysfunction, infection, or cachexia. The disease is often misdiagnosed if there is no family history to increase clinical suspicion. The diagnosis can be confirmed by histopathology and genetic analysis.

2.2. Analysis of Current Treatment Options

There is no approved drug for hATTR amyloidosis in the United States. Treatment options for hATTR amyloidosis include liver transplant and medical management of associated symptoms. For neuropathy, management includes medications such as gabapentin to alleviate paresthesias. Although not approved for hATTR amyloidosis, the nonsteroidal anti-inflammatory drug diflunisal is sometimes used off-label to treat the disease. Tafamidis has been approved in Europe for hATTR-PN. Some patisiran study subjects used tafamidis and diflunisal as concomitant medications.

3. Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Patisiran is a new molecular entity (NME) that is not currently marketed in the United States or in any other country.

3.2. Summary of Presubmission/Submission Regulatory Activity

The FDA granted patisiran Orphan Drug (6/14/2012) and Fast Track (10/31/2013) designations.

A Pre-IND meeting was held on 3/14/13. There was a discussion of the overall CMC information, nonclinical plan, and the preliminary clinical data that were intended to support the inclusion of U.S. sites and initiation of dosing with respect to an ongoing Phase 2 study. During this meeting, there was also a discussion of the number of patients planned for enrollment in the US, adequacy of the number of patients per cohort, nonclinical data needed to support open-label extension studies, and suicidality monitoring. The associated IND 117395 became active on 5/29/2013.

Breakthrough Therapy designation was denied on 8/7/2013, because the preliminary evidence submitted was limited and did not indicate that patisiran may demonstrate substantial improvement over existing therapies on one or more clinically significant endpoints.

On 9/23/2013, an End-of-Phase 2 meeting was held to discuss the overall development plan for patisiran and the design of a proposed Phase 3 clinical protocol.

A Type C guidance meeting was held on 10/8/2015, to discuss an interim analysis plan for the Phase 3 trial. There was discussion of the modified Neurologic Impairment Score +7 (mNIS+7) as the primary endpoint with Norfolk Quality of Life – Diabetic Neuropathy Score (Norfolk QOL-DN) as a key secondary endpoint. The sponsor ultimately did not conduct the interim analysis in its Phase 3 trial because of unexpectedly fast study recruitment.

A topline results summary of the Phase 3 (APOLLO) study was submitted on 9/17/2017, reporting statistically significant positive results for all endpoints.

On September 29, 2017, the sponsor submitted requests for rolling submission review and Breakthrough Therapy designation, which were granted by the Agency on October 19, 2017, and November 17, 2017, respectively.

A Pre-NDA meeting was held on 11/13/2017, to seek Agency feedback on the timing,

content, and format of elements of a rolling NDA submission.

The application submission date was 12/11/2017.

3.3. Foreign Regulatory Actions and Marketing History

Patisiran is not approved in any country.

4. Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

See the OSI report for a full discussion of site inspections and specific findings. The OSI report concludes that objectionable conditions or practices that are significant deviations from Good Clinical Practice (GCP) were noted at the sponsor and CRO inspections, but that the findings are unlikely to impact data reliability significantly.

Note that the European Medicines Agency (EMA), which had received the same marketing authorization application for patisiran, identified critical inspectional findings at Site #61, including deficiencies in the informed consent process, in preserving the confidentiality of subjects, in the management of investigational product, and in source document management. Major findings included lack of GCP training documentation, some ICF versions lacking signature option of legal representative of the subject or a witness, protocol deviations, and late reporting of SAEs.

OSI therefore recommended that a statistical sensitivity analysis excluding the data from Site #61 be conducted. This sensitivity analysis was done and exclusion of Site #61 did not significantly affect the primary efficacy assessment.

4.2. Product Quality

Patisiran (ALN-TTR02; patisiran-LNP) is a ribonucleic acid (RNA) interference (RNAi) therapeutic product comprised of 2 mg/mL patisiran drug substance (ALN-18328) and lipid excipients DLin-MC3-DMA, DSPC, cholesterol, and PEG2000-C-DMG as lipid nanoparticles (LNPs) in isotonic phosphate buffered saline.

Analysis and discussion of the acceptability of product quality is deferred to the chemistry, manufacturing, and controls (CMC) reviewer.

4.3. Clinical Microbiology

Not applicable.

4.4. Nonclinical Pharmacology/Toxicology

The applicant reports the following results of nonclinical studies. The reader is referred to the separate nonclinical review for further details and analysis.

Pharmacology studies in monkeys demonstrated reduction in serum TTR levels (98%), serum levels of RBP (60%), vitamin A (90%), and T4 (41%) at the 0.3 mg/kg q3w dose. After 9 months of dosing in the chronic toxicology study in monkeys, there were no abnormalities in thyroid histopathology, ophthalmic examination findings, electroretinograms, or ocular histopathology findings. In a safety pharmacology studies in monkeys, there were no observed effects on the ECG parameters (including QT intervals) and mean arterial blood pressure at ≤6 mg/kg. Heart rate and body temperature were increased at ≥3 mg/kg. Respiratory parameters and neurological examinations were not affected at 3 mg/kg, the only dose evaluated.

In rat studies, there were mild to moderate increases in serum liver markers (alanine transaminase [ALT], AST, alkaline phosphatase, and/or total bilirubin). Histopathology findings (hepatocellular/single cell necrosis, inflammation, pigment deposition, and/or monocytic infiltration) were observed at >0.1 mg/kg in rats and >1.0 mg/kg in monkeys. In the spleen, lymphoid atrophy/necrosis and histiocytosis in the white pulp were observed in rats and hypocellularity of the red pulp was observed in monkeys.

The FDA nonclinical review concluded that “chronic toxicity studies indicated a risk for liver injury based on findings in rats and monkeys. However, such findings are reversible and can be clinically monitored.”

4.5. Clinical Pharmacology

The main conclusions of the clinical pharmacology discipline review are summarized below. The reader is referred to the full review of the Office of Clinical Pharmacology (OCP) for further details.

Clinical Pharmacology Review Summary	Recommendations and Comments

General dosing instructions	The proposed dosing regimen of patisiran is 0.3 mg/kg IV infusion over 80 min every 3 weeks. For patients weighing ≥100 kg, the dose is capped to 30 mg.
Dosing in patient subgroups (intrinsic and extrinsic factors)	No dose adjustments are required. Hepatic/renal impairment is not expected to affect patisiran exposures. Drug interaction liability with patisiran is considered low.
Labeling	The clinical pharmacology labeling concepts proposed by the applicant are generally adequate.
Bridge between the to-be-marketed and clinical trial formulations	The to-be-marketed formulation is the same as the one used in the pivotal efficacy study.

4.5.1. Mechanism of Action

In hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis), misfolding of transthyretin (TTR) protein leads to its aggregation and the formation of amyloid fibrils. Patisiran (Onpattro™; called ALN-TTR02 in clinical trials) is designed to treat the disease by reducing the amount of TTR protein production.

Patisiran is a small interfering ribonucleic acid (siRNA) that targets TTR messenger ribonucleic acid (mRNA). The mechanism of action of patisiran is via ribonucleic acid interference (RNAi). RNAi is a biological process by siRNA, typically 21-23 nucleotides in length, which can direct sequence-specific degradation of mRNA. When synthetic siRNAs are introduced into cells, the net effect is the binding of the siRNA to its complementary mRNA sequence, the cleavage of this target mRNA, and the suppression of the target protein encoded by the mRNA. Thus, patisiran suppresses the production of TTR (both wild type and mutated type).

Since unformulated siRNAs are rapidly eliminated and do not achieve significant tissue distribution, siRNA targeting TTR mRNA are delivered to target tissue in lipid nanoparticle (LNP) formulations intravenously. The target tissue is primarily the liver.

4.6. Devices and Companion Diagnostic Issues

Not applicable to this application.

4.7. Consumer Study Reviews

Not applicable to this application.

5. Sources of Clinical Data and Review Strategy

5.1. Table of Clinical Studies

The studies submitted to support the safety and efficacy of patisiran are summarized in the table below.

Table 1: Listing of Clinical Trials Relevant to this NDA, copied from submission.

Study Identifier	Type of Study and Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects or Patients and Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Location: 5.3.3.1						
ALN-TTR02-001	PK Primary: Evaluate the safety and tolerability of patisiran-LNP Secondary: Characterize the PK of patisiran-LNP and assess the PD effect of patisiran-LNP on TTR, vitamin A, and RBP	Phase 1, randomized, single-blind, placebo-controlled, SAD	Patisiran-LNP (ALN-TTR02); 0.01, 0.05, 0.15, 0.3 and 0.5 mg/kg IV infusion	17 Placebo: n=4 Patisiran-LNP: n=13 Healthy Subjects	Single dose (Day 0); monitored for safety, PK and PD up to 180 days post dose	Complete; Full
ALN-TTR02-005	PK Primary: Evaluate the safety and tolerability of patisiran-LNP when administered to Japanese healthy volunteers Secondary: Characterize the PK of patisiran-LNP and assess the PD effect of patisiran-LNP on TTR, vitamin A, and RBP	Phase 1, randomized, double-blind, placebo-controlled SAD	Patisiran-LNP; 0.05, 0.15 and 0.3 mg/kg; IV infusion	12 Placebo: n=3 Patisiran-LNP: n=9 Healthy Subjects	Single dose (Day 0); monitored for safety, PK and PD up to 90 days post dose	Complete; Full

Study Identifier	Type of Study and Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects or Patients and Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Location: 5.3.4.2						
ALN-TTR02-002	PK/PD Primary: Evaluate the safety and tolerability of patisiran-LNP Secondary: Characterize the PK of patisiran-LNP and assess of the PD effect of patisiran-LNP on TTR	Phase 2, open-label, MAD	Patisiran-LNP(ALN-TTR02); 0.01, 0.05, 0.15, 0.3 mg/kg q4w as well as 0.3 mg/kg q3w; IV infusion	29 (all treated with patisiran-LNP) Patients with hATTR amyloidosis with polyneuropathy	2 doses (Day 0 and Day 21) - q3w 2 doses (Day 0 and Day 28) - q4w monitored for safety, PK and PD up to 208 days postdose.	Complete; Full
Location: 5.3.5.1						
ALN-TTR02-004	Efficacy/Safety Primary: Evaluate the difference between the patisiran-LNP and placebo groups in the change from baseline of mNIS+7 score at 18 months Secondary: Determine the effect of patisiran-LNP, based on the change from baseline at 18 months, on Norfolk QOL-DN, NIS-weakness, R-ODS score, 10-meter walk test (gait speed), mBMI, and COMPASS-31	Phase 3 randomized, double-blind, placebo-controlled	Patisiran-LNP(ALN-TTR02); 0.3 mg/kg q3w; IV infusion	225 Placebo: n=77 Patisiran-LNP: n=148 Patients with hATTR amyloidosis with polyneuropathy	18 months Follow-up for all patients for 21 days after last dose. Patients who did not enroll in Study ALN-TTR02-006 had an additional follow-up at 56 days after last dose.	Complete; Full

Study Identifier	Type of Study and Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects or Patients and Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Location: 5.3.5.2						
ALN-TTR02-003	Efficacy/Safety Primary: Evaluate the safety of long-term dosing with patisiran-LNP Secondary: Assess the PD effect long-term dosing of patisiran-LNP on TTR and assess changes from baseline in mNIS+7 score, EQ-5D and R-ODS, gait speed, grip test, and mBMI	Phase 2, multicenter, single-arm, open-label, extension study in patients previously participating in Study ALN-TTR02-002	Patisiran-LNP (ALN-TTR02); 0.3 mg/kg q3w; IV infusion	27 dosed 25 completed Patients with hATTR amyloidosis with polyneuropathy	2 years; Follow-up 21 days after last dose. Only the patients who didn't roll over to the 006 study also had a follow up at 56 days after last dose.	Complete; Full
ALN-TTR02-006	Efficacy/Safety Primary: Assess the safety and efficacy of long-term dosing with patisiran-LNP	Phase 3 multicenter, open-label, extension study in patients who completed Study ALN-TTR02-003 and Study ALN-TTR02-004	Patisiran-LNP (ALN-TTR02); 0.3 mg/kg, q3w; IV infusion	188 enrolled 184 treated (All treated with patisiran-LNP) Patients with hATTR amyloidosis with polyneuropathy	Up to 5 years	Ongoing Interim – data cutoff date 14 July 2017

Study Identifier	Type of Study and Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects or Patients and Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Location: 5.3.5.4						
ALN-TTR02-007	Safety Primary: Assess the long-term safety of Patisiran-LNP Secondary: Assess effect of Patisiran-LNP on serum TTR levels and polyneuropathy progression	Open-label, multicenter,expanded access	Patisiran-LNP (ALN-TTR02); 0.3 mg/kg q3w; IV infusion	Up to 150 planned Patients with hATTR Amyloidosis with Polyneuropathy	Until commercially available in the US	Ongoing expanded access. Documents being provided are limited to protocol, data listings, and limited number of summary tables.

Abbreviations: hATTR amyloidosis=hereditary transthyretin-mediated amyloidosis; IV=intravenous; MAD=multiple-ascending dose; mNIS+7=Modified Neuropathy Impairment Score +7; PD=pharmacodynamics; PK=pharmacokinetics;q3w=once every 3 weeks; q4w=once every 4 weeks; RBP=retinol binding protein; SAD=single-ascending dose; TTR=transthyretin

5.2. Review Strategy

Effectiveness and safety were assessed by evaluating the results from the randomized, double-blind, placebo-controlled Phase 3 trial (ALN-TTR02-004, APOLLO, "Study 004") in patients with hATTR amyloidosis with polyneuropathy in addition to supportive open-label studies (ALN-TTR02-003, -006, and -007). The effectiveness assessment focused on the clinical interpretability of the trial endpoints and the applicant's reported results. Confirmation of the efficacy analyses themselves was provided by the biometrics reviewer for this application. The safety assessment was based on the applicant's reports and clinical reviewer analysis of the submitted data.

6. Review of Relevant Individual Trials Used to Support Efficacy

6.1. ALN-TTR02-004, APOLLO

6.1.1. Study 004 Design

Overview and Objective

Study 004 was a Phase 3, multinational, randomized, double-blind, placebo-controlled study of patisiran in subjects with polyneuropathy caused by hATTR amyloidosis.

The objectives of the study are described by the applicant as follows:

- The primary objective of the study was to determine the efficacy of patisiran-LNP (ALN--TTR02) by evaluating the difference between the patisiran-LNP and placebo groups in the change from baseline of mNIS+7 score at 18 months.

- The secondary objectives of the study were to determine the effect of patisiran-LNP on various clinical parameters by assessing the difference between patisiran-LNP and placebo in the change from baseline in the following measurements at 18 months:
    - Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) questionnaire;
    - Neurological impairment score (NIS)-weakness (NIS-W) score;
    - Rasch-built Overall Disability Scale (R-ODS) score;
    - Timed 10-meter walk test (10-MWT, gait speed);
    - Modified body mass index (mBMI);
    - Autonomic symptoms questionnaire (Composite Autonomic Symptom Score [COMPASS 31]).

- The exploratory objectives of the study were to determine the difference between the patisiran-LNP and placebo groups in the change from baseline in the following measurements at 18 months:
  - NIS+7 score;
  - Grip strength;
  - EuroQOL (EQ-5D) questionnaire;
  - Large vs small nerve fiber function including nerve conduction studies sum of 5 attributes (NCS Σ5), quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), vibration detection threshold (VDT), heart rate variability to deep breathing (HRdb), postural blood pressure;
  - Pathologic evaluation of sensory and autonomic innervation through voluntary skin punch biopsies and analysis of intraepidermal nerve fiber density (IENFD), sweat gland nerve fiber density (SGNFD), and dermal amyloid content (assessment of dermal amyloid content was added through a Global Administrative Letter, see Section 9.8.1);
  - Assessment of ambulation through FAP stage and Polyneuropathy Disability (PND) score;
  - Cardiac assessment through echocardiogram, troponin I, and N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) levels;
  - Pharmacodynamic (PD) biomarkers [TTR, retinol binding protein (RBP), vitamin A];
  - To compare the proportion of patients in the patisiran-LNP and placebo groups who met the pre-defined criterion for rapid disease progression (defined as ≥24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline) at 9 months.
  - To serially evaluate lower limb nerve injury via voluntary magnetic resonance (MR) neurography approximately every 6 months in patients receiving either patisiran-LNP or placebo from France and Germany.

Trial Design

- **Basic study design:**
  This was a multicenter (44 study centers), multinational (19 countries), randomized, double-blind, placebo-controlled, Phase 3 study. The planned study size was N=200; the actual number randomized in this study was N=225.

  The duration of patient participation in this study was approximately 21 months including Screening, On-Treatment (Day 0 to Week 79-80 or Month 18), and Follow-up periods (Week 81 to Week 86).

- **Choice of control group:**

  A double-blinded placebo-control study is a rigorous and readily interpretable study design. It is ethically acceptable for this indication given the unknown effect of the study drug and the lack of any effective treatment that might otherwise have served as an active control. Placebo group patients continued to receive the standard of care for hATTR amyloidosis.

- **Key inclusion/exclusion criteria:**

  The criteria listed below from the submitted protocol appear adequate to enroll adult patients with hATTR amyloidosis with polyneuropathy representative of the U.S. population.

Key Inclusion Criteria
To participate in this study, candidates were required to meet the following eligibility criteria at Screening:

1. Male or female of 18 to 85 years of age (inclusive);
2. Had a diagnosis of FAP with documented TTR mutation;
3. Had an NIS of 5 to 130 (inclusive) and a PND score of ≤3b
4. Had an NCS sum of the sural sensory nerve action potential (SNAP), tibial compound muscle action potential (CMAP), ulnar SNAP, ulnar CMAP, and peroneal CMAP of ≥2 points
5. Had a Karnofsky performance status of ≥60%;
6. Must have been willing and able to comply with protocol-required visit schedule and visit requirements and provide written informed consent.

Key Exclusion Criteria
Subjects meeting any of the following criteria were not eligible for the study:

1. Had a prior liver transplant or was planning to undergo liver transplant during the study period;
2. Had other known causes of sensorimotor or autonomic neuropathy (e.g., autoimmune disease, monoclonal gammopathy, etc.);
3. Had known primary amyloidosis or leptomeningeal amyloidosis;
4. Had known type I diabetes;
5. Had had type II diabetes mellitus for ≥5 years;
6. Had vitamin B12 levels below the lower limit of normal (LLN);
7. Had a New York Heart Association heart failure classification >2;

8. Had acute coronary syndrome within the past 3 months;
9. Had uncontrolled cardiac arrhythmia or unstable angina;
10. Had a known history of alcohol abuse within the past 2 years or daily heavy alcohol consumption (females: more than 14 units of alcohol per week; males: more than 21 units of alcohol per week [unit: 1 glass of wine [125 mL] = 1 measure of spirits = ½ pint of beer]);
11. Was currently taking diflunisal; if previously on this agent, must have had at least a 3-day wash-out prior to start of study drug administration in this study;
12. Was currently taking tafamidis, doxycycline, or tauroursodeoxycholic acid; if previously on any of these agents, must have completed a 14-day wash-out prior to start of study drug administration in this study;
13. Was under legal protection (defined as "any person who becomes incapable of protecting his/her interests due to a medically diagnosed impairment of his/her mental faculties that may limit or prevent the expression of his/her will").

- **Dose selection:**
  The dose level for this clinical study, 0.3 mg/kg every 3 weeks as an IV infusion, was selected based on the results of the Phase 2 multiple ascending-dose (MAD) study (Study ALN TTR02 002) in which 29 patients with hATTR amyloidosis with polyneuropathy received 2 doses of patisiran-LNP ranging from 0.1 mg/kg to 0.3 mg/kg administered every 3 weeks (q3w) or every 4 weeks (q4w). A dose-dependent reduction of serum TTR was observed with reductions >80% noted at the patisiran-LNP dose of 0.3 mg/kg. TTR reduction of >80% was better maintained when patisiran-LNP was administered q3w than q4w.

- **Study treatments:**
  Patients who were randomized into the active treatment group received 0.3 mg/kg patisiran-LNP every 3 weeks diluted in 0.9% sodium chloride solution (normal saline). Patients who were randomized into the control group received placebo (normal saline) every 3 weeks.

  All patients received premedication in order to reduce the potential of an infusion-related reaction (IRR), as described in more detail in Section 7.1.4. Patients received the following on the day of study drug administration at least 60 min prior to the patisiran-LNP infusion: IV dexamethasone (10 mg) or equivalent, oral paracetamol or acetaminophen (500 mg) or equivalent, IV H2 blocker (e.g., ranitidine 50 mg, famotidine 20 mg, or equivalent other H2 blocker dose) and IV H1 blocker diphenhydramine 50 mg.

  Please refer to the Office of Pharmaceutical Quality (OPQ) review for discussion of the product formulation used for the active study arm.

- **Assignment to treatment:**
  Patients were randomly assigned in a 2:1 ratio to receive either 0.3 mg/kg patisiran-LNP or placebo. The randomization was stratified by three factors: Baseline NIS (5-49 vs 50-130), early onset V30M (<50 years of age at onset) vs all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs no previous tetramer stabilizer use.

  Patients were randomized on Visit Day 0 (predose) via an interactive response or voice system (IRS/IVRS). Only the pharmacist or unblinded personnel were allowed to receive the treatment code.

- **Blinding:**
  The following methods were used for blinding. These methods appear adequate.

  All site personnel were blinded to the study treatment, except the pharmacist and designated site personnel who set-up, dispensed, and prepared the infusion.

  All infusion bags and lines were covered with amber bags and line covers by the unblinded personnel.

  All patients were blinded to study drug assignment and received an IV infusion q3w using identical volumes for patisiran-LNP and placebo.

  Blinded study personnel who performed assessments related to efficacy endpoints were separate from personnel who monitored the administration of study drug.

- **Administrative structure:**
  Nineteen countries and 44 study centers worldwide in North America, Europe, Asia, and South America, randomized patients in this study. Investigators were trained at site initiation visits.

  An independent Data Monitoring Committee (DMC) was involved in the conduct of this study.

  An independent Clinical Adjudication Committee was employed to determine if at the Month 9 assessment visit, a patient exhibited rapid disease progression (defined as ≥24-

point increase in modified Neurologic Impairment Score +7 (mNIS+7) from baseline and Familial Amyloidotic Polyneuropathy (FAP) stage progression relative to baseline.

The Clinical Endpoints Adjudication Committee was an independent committee asked to attribute cause of death according to the responsible underlying disease process.

- **Procedures and schedule:**
  The schedule of assessments from Screening to the 9-Month Assessment, Week 39 to Week 86/Early Termination are presented in the following tables, copied from the submission.

Table 2: Schedule of Assessments: Screening to 9-Month Efficacy Assessment. Source: Study 004 CSR, p. 40

Procedure	Screening [a]	Screening/ Baseline [a]	Baseline [a]	Predosing	Dosing													9-Month Efficacy Assessment [y]
Study Day	Day -42 to 0			D0 Predose	D0	D21	D42	D63	D84	D105	D126	D147	D168	D189	D210	D231	D252	D253-D272
Study Week	NA			0	0	3	6	9	12	15	18	21	24	27	30	33	36	36-39
Windows	NA			0	0	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	NA
Informed Consent	X																	
Inclusion/Exclusion Criteria	X	X [a]																
Medical History	X	X [b]	X [b]															
Demographics	X																	
Review Documentation of TTR Genotype	X																	
HIV Status Review	X																	
Karnofsky Performance Status	X																	
New York Heart Classification	X																	
Serology Testing [c]	X																	
Paraprotein by IFE	X																	
Vitamin B12	X																	
Efficacy Assessments																		
NIS [d] / NCS [d]	X																	
mNIS+7 [e]		X	X															X X
NIS+7 [f]		X	X															X X
PND Score and FAP Stage		X [g]	X															X
Skin Punch Biopsy (IENFD and SGNFD) [h]			X															X
mBMI [i]			X															X
10-meter Walk Test [j]		X [z]																X X
Grip Strength Test [k]		X [z]																X X
Norfolk QOL-DN; COMPASS 31 Questionnaires		X [z]																X
EQ-5D; R-ODS Questionnaires		X [z]																X
Echocardiogram		X [z]																X
NT-proBNP and Troponin I			X															X
MR Neurography [aa]			~EVERY 6 MONTHS FOR STUDY DURATION															
Pharmacodynamic Assessments [l]																		
TTR Protein, Vitamin A, and RBP			X	X		X					X						X	X
Obtain Blood Sample for Long-term Storage		X	X	X		X					X						X	X

	Visit Type	Screening [a]	Screening/ Baseline [a]	Baseline [a]	Predosing	Dosing													9-Month Efficacy Assessment[y]
	Study Day	Day -42 to 0			D0 Predose	D0	D21	D42	D63	D84	D105	D126	D147	D168	D189	D210	D231	D252	D253-D272
	Study Week	NA			0	0	3	6	9	12	15	18	21	24	27	30	33	36	36-39
Procedure	Windows	NA			0	0	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	NA
Safety Assessments [m]																			
Physical Examination		X																X	
Weight [n]		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X X
Height		X																	
Vital Signs [o]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X X
12-Lead ECG [p]			X [z]									X							X
Serum Chemistry		X			X					X					X				
Hematology, Urinalysis		X																X	
Thyroid Function Tests		X																X	
Coagulation Studies		X																	
Antidrug Antibody Testing [q]					X		X					X						X	
Pregnancy Test [r]		X																	
Ophthalmology Exam [s]					X													X	
Concomitant Medications		X	X	X								X							
Adverse Events												X							
Pharmacokinetic Assessments																			
Plasma PK Sampling [t]					X	X	X					X						X	
Urine PK Sampling [u]					X		X					X						X	
Other Assessments																			
Pharmacoeconomic Questionnaire					X [z]														X
C-SSRS Questionnaire			X [z]																X
Drug Administration																			
Randomization [v]					X														
Premedication Administration [w]					X		X	X	X	X	X	X	X	X	X	X	X	X	
Study Drug Administration [x]						X	X	X	X	X	X	X	X	X	X	X	X	X	

Abbreviations: COMPASS 31=Composite Autonomic Symptom Score; EQ-5D=EuroQOL-5 Dimensions; ECG=electrocardiogram; FAP=familial amyloidotic polyneuropathy; HIV=human immunodeficiency virus; IENFD=Intraepidermal nerve fiber density; IFE=immunofixation electrophoresis; mBMI=modified body mass index; mNIS=Modified Neuropathy Impairment Score; NCS=nerve conduction studies; NIS=Neuropathy Impairment Score; NT-proBNP=N-terminal prohormone of B-type natriuretic peptide; PK=Pharmacokinetics; PND=polyneuropathy disability; QOL-DN=Quality of Life-Diabetic Neuropathy; RBP=retinol binding protein; R-ODS=Rausch-built Overall Disability Scale; SGNFD=Sweat gland nerve fiber density; TTR=transthyretin.

a. The Screening/Baseline and Baseline visits were performed on separate days. The Screening/Baseline visit was performed within 21 days prior to the first dose of study drug (Day 0). The Baseline visit was conducted at least 24 hours (approximately), but not more than 7 days, after the Screening/Baseline visit. In conjunction with the decision of the Medical Monitor(s), patients were allowed to rescreen after a minimum of 5 days had elapsed from their last screening assessment. Note: Inclusion Criteria 3 (ie, NIS of 5 to 130 [inclusive] and PND score ≤3b) and 4 (ie, NCS sum of the sural sensory nerve action potential [SNAP], tibial compound muscle action potential [CMAP], ulnar SNAP, ulnar CMAP, and peroneal CMAP of ≥2 points) were to be met at the Screening/Baseline visit. All other entry criteria (inclusion and exclusion) were assessed at the Screening visit only.
b. An interval medical history was collected at the Screening/Baseline and Baseline visit. Only changes since the Screening visit were collected.
c. Serologies included hepatitis B surface antibody (HbsAb), hepatitis B surface antigen (HbsAg), and anti–hepatitis C virus antibody (anti-HCV Ab).
d. The NIS and NCS were assessed for the likelihood of a patient meeting the NIS and NCS eligibility criteria at the Screening/Baseline visit. The documented results of previously performed NIS and NCS may have been used to qualify a patient for this study if these tests were performed within 60 days prior to the date of informed consent.
e. The mNIS+7 consisted of the modified NIS tool (weakness and reflexes), NCS 5 attributes (Σ5), quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), and postural blood pressure. At the 9-month efficacy assessment, 2 assessments of the mNIS+7 were performed on separate days (1 assessment on each day). The second (repeat) assessment was conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart. Components that were shared between the mNIS+7 and NIS+7 (including NIS and NCS) were performed once at each assessment (eg, the weakness component was not performed more than once on any given day).
f. The NIS+7 consisted of the NIS tool (weakness, sensation, and reflexes), NCS Σ5, vibration detection threshold (VDT), and heart rate response to deep breathing (HRdB). At the 9-month efficacy assessment, 2 assessments of the NIS+7 were performed on separate days (1 assessment on each day). The second (repeat) assessment was conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart. Components that were shared between the mNIS+7 and NIS+7 (including NIS and NCS) were performed once at each assessment (eg, the weakness component was not performed more than once on any given day).
g. At the Screening/Baseline visit, only PND score was required.
h. If the patient had provided separate informed consent for skin biopsies, 2 sets of tandem 3-mm skin punch biopsies were obtained (4 biopsies total). One set of biopsies was taken from the distal lower leg, when a patient's clinical status allowed, and one set from the distal thigh at each time point. Skin biopsies were performed at a central assessment site (CAS).
i. mBMI was calculated programmatically in the clinical database; the site did not perform the calculation.
j. The patient was asked to walk 10 meters. The walk was to be completed without assistance from another person; ambulatory aids such as canes and walkers were permitted. The time required for the patient to walk 10 meters was recorded. At the 9-month efficacy assessment, 2 assessments of the 10-meter walk test were performed on separate days (1 assessment on each day). The second (repeat) assessment was conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart.
k. Grip strength was measured in triplicate using a dynamometer held in the dominant hand. Every effort was made to use the same device for a patient throughout the duration of the study. At the 9-month efficacy assessment, 2 assessments of the grip strength were performed on separate days (1 assessment on each day). The second (repeat) assessment was conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart.
l. On dosing days, blood samples for PD assessments were obtained prior to dosing and vitamin A supplementation.
m. On dosing days, all safety assessments were performed predose.
n. Weight from previous visit was used for calculating dose. Weight was collected predose.
o. Vital signs included: blood pressure, pulse rate, temperature, and respiratory rate. Parameters were measured in the supine position using an automated instrument after the patient had rested comfortably for 10 minutes. Vital signs were collected predose. On Day 0, vital signs were also collected postdose.
p. All ECGs were obtained in triplicate.
q. Blood samples for antidrug antibody testing were collected prior to study drug dosing.
r. A pregnancy test (urine- or serum-based) was performed on all females of child-bearing potential.

s. The baseline ophthalmology examination was performed any time after the patient was deemed eligible for participation in the study through Day 21. The 9-month ophthalmology examination was performed between Days 231(±3) and 272 at a CAS.
t. Plasma PK samples were collected as follows: Day 0: predose (within 1 hour of planned study drug dosing) and at the end of infusion (+5 minutes). Day 21 and Day 252: predose (within 1 hour of planned study drug dosing) and 30 minutes after the end of the infusion (+15 minutes). Day 126: pre-dose (within 1 hour of planned study drug dosing) and at the end of infusion (+5 minutes).
u. Urine PK samples were collected pre-dose (within 1 hour of planned study drug dosing).
v. Randomization procedures are described in protocol Section 4.4.1.
w. Prior to dosing, all patients received premedications administered at least 60 minutes prior to the start of infusion of study drug. The regimen is described in protocol Section 5.3.3.
x. The patient's infusion site was assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion. The patient remained at the study site for 1 hour following completion of dosing for observation and completion of assessments.
y. Patients who discontinued study drug due to rapid disease progression based on the 9-month efficacy assessments were to continue on to the Modified Visit Schedule (See Section 9.3.3.2).
z. Assessments were completed at a single time point during one of the specified visits, at the discretion of the Investigator.
aa. For patients from France and Germany only (Added per Country-specific Amended version 5.2 and 5.1, respectively): Participation was voluntary; patients who elected to undergo MR neurography provided informed consent prior to first assessment. For patients who had already begun the study and who subsequently provided consent for MR neurography, the baseline assessment was performed soon after the consent followed by serial assessments at approximately 6-month intervals thereafter for the study duration.

Table 3: Schedule of Assessments: Week 39 to Week 86/Early Withdrawal. Source: Study 004 CSR, p. 44.

Procedure																								
Visit Type	Dosing														18-Month Efficacy Assessment		21-Day Follow-up End of Study(a)	56-Day Follow-up(a)	Early Withdrawal(b)		Follow-up for Patients who Discontinue Treatment but Return at 9 and/or 18 mo(c)			
Study Day	D273	D294	D315	D336	D357	D378	D399	D420	D441	D462	D483	D504	D525	D546	D553-D560		D567	D602	NA		D253-D272		D553-D560	
Study Week	39	42	45	48	51	54	57	60	63	66	69	72	75	78	79-80		81	86	NA		36-39		79-80	
Windows	+3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	NA		±7D	±10D	2D to 7D		±10D		±10D	
Efficacy Assessments																								
mNIS+7(d)															X	X			X	X	X	X	X	X
NIS+7(e)															X	X			X	X	X	X	X	X
PND Score and FAP Stage															X				X		X		X	
Skin Punch Biopsy (IENFD and SGNFD)(f)															X				X					
mBMI(g)															X				X		X		X	
10-meterWalkTest(h)															X	X			X	X				
Grip Strength Test(i)															X	X			X	X				
Norfolk QOL-DN; EQ-5D; R-ODS Disability; COMPASS31 Questionnaires															X				X		X(j)		X(j)	
Echocardiogram															X				X					
NT-pro BNP and Troponin I															X				X					
MR Neurography(x)	~EVERY 6 MONTHS FOR STUDY DURATION																							
Pharmacodynamic Assessments(k)																								
TTR Protein, Vitamin A, and RBP	X						X							X	X		X		X		X		X	
Obtain Blood Sample for Long-term Storage	X						X							X	X		X		X					
Safety Assessment(l)																								
Physical Examination														X			X	X	X		X		X	
Weight(m)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X		X	
Vital Signs(n)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				

Procedure	Dosing														18-Month Efficacy Assessment	21-Day Follow-up End of Study[a]	56-Day Follow-up[a]	Early Withdrawal[b]	Follow-up for Patients who Discontinue Treatment but Return at 9 and/or 18 mo[c]	
Study Day	D273	D294	D315	D336	D357	D378	D399	D420	D441	D462	D483	D504	D525	D546	D553-D560	D567	D602	NA	D253-D272	D553-D560
Study Week	39	42	45	48	51	54	57	60	63	66	69	72	75	78	79-80	81	86	NA	36-39	79-80
Windows	+3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	NA	±7D	±10D	2D to 7D	±10D	±10D
12-Lead ECG[o]							X								X			X		
Serum Chemistry					X					X				X			X	X		
Coagulation[w]														X						
Hematology, Urinalysis														X				X		
Thyroid Function Tests														X				X		
Antidrug Antibody Testing[p]							X							X				X		
Pregnancy Test[q]																X	X	X		
Ophthalmology[r]															X					
Concomitant Medications											X									
Adverse Events										X										
Pharmacokinetic Assessments																				
Plasma Pharmacokinetic Sampling[s]							X							X				X	X	X
Urine Pharmacokinetic Sampling[t]							X							X				X		
Other Assessments																				
Pharmacoeconomic Questionnaire															X			X		
C-SSRS Questionnaire															X			X		
Drug Administration																				
Premedication Administration[u]	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
Study Drug Administration[v]	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
Karnofsky performance status																			X	X
NYHA class																			X	X

w. INR assessment only, which was used for qualification for Study ALN-TTR02-006.
x For patients from France and Germany only (Added per Country-specific Amended version 5.2 and 5.1, respectively): Participation was voluntary; patients who elected to undergo MR neurography provided informed consent prior to first assessment. For patients who had already begun the study and who subsequently provided consent for MR neurography, the baseline assessment was performed soon after the consent followed by serial assessments at approximately 6-month intervals thereafter for the study duration.

Rainer – please try to avoid these. No one wants to read this; on one can read the footnotes. Try to make your own condensed table that includes only the salient information – on one page or less, with few if any footnotes. You can do this in excel.

- **Dietary restrictions/instructions:**
  Patients should have started vitamin A supplementation by the time they start the first dose of patisiran or placebo.

  See the discussion of potentially low vitamin A levels caused by patisiran in Section 8.5.5.

- **Concurrent medications:**
  Use of the following medications/treatments was prohibited during study participation (excluding patients who had rapid disease progression and discontinued study drug after the 9-month efficacy assessments in which case local standard of care treatment

for hATTR amyloidosis with polyneuropathy such as tetramer stabilizers was allowed):

- Any investigational agent other than patisiran-LNP
- Tafamidis
- Diflunisal
- Doxycycline/TUDCA
- Corticosteroids other than those administered as premedications prior to the dose of patisiran-LNP, those used to treat an infusion reaction, or topical or inhaled corticosteroids. However, for patients with chronic inflammatory disorders (e.g., asthma, rheumatoid arthritis, etc.), systemically administered steroids were permitted provided that: 1) the dose was <20 mg/day prednisone or equivalent if administered chronically, or 2) for doses ≥20 mg/day, administration was limited to no more than 5 consecutive days. Additionally, an intra-articular injection of a corticosteroid was permitted.

Medications and treatments other than those specified above, including palliative and supportive care approved by the Investigator for disease-related symptoms, as well as herbal preparations, over-the-counter (OTC) medications, vitamins, and minerals, were permitted during the study.

Patients received an oral daily supplemental dose of the recommended daily allowance of vitamin A.

- **Treatment compliance:**
  Treatment compliance with study drug administration was verified by unblinded study staff observation.

  A dose was considered completed if 80% or more of the total volume of the IV solution was administered to the patient. If a patient missed 2 consecutive doses, the PI, in consultation with the Medical Monitor, discussed whether the patient was able to continue on the study. Study drug delay was recorded in the case report forms (CRF) when a scheduled infusion cycle was missed or delayed out of window as a result of an AE.

  The Investigator maintained records of receipt and the condition of all study drugs including dates of receipt. Records were kept of how much study drug was dispensed and used by each patient in the study.

  Allowing occasional missed doses is acceptable. A dose delay beyond the effective half-life based on accumulation of 22 days could allow drug concentrations to drop below therapeutic levels and might diminish efficacy.

- **Rescue medication:**
  There were no applicable rescue medications for this trial.

  Note that infusion-related reactions from patisiran have the potential risk of anaphylaxis, which can be treated with epinephrine injection. No anaphylaxis occurred in the patisiran clinical studies.

- **Subject completion, discontinuation, or withdrawal:**
  A patient was considered to have completed study treatment if they had completed the drug regimen without permanently stopping treatment prior to the last dose at the Week 78 visit. A patient was considered to have completed the study if the patient did not withdraw consent from the study, and completed protocol-specified procedures up through the 18-month efficacy assessment visit (Week 79-80).

  There were 3 ways for a patient to discontinue study drug and/or withdraw from the study:

  1) The patient or Investigator decided to discontinue study drug, but the patient agreed to remain in the study and undergo follow-up assessments
  2) The patient experienced protocol defined rapid disease progression at Month 9 and elected to discontinue study drug but remain in the study and return for protocol-specified visits under a modified schedule of assessments, including follow-up assessment at Month 18.
  3) The patient decided to no longer participate in the study and withdrew consent (Study 004 CSR, p. 31).

Study Endpoints

Primary Efficacy Endpoint

The primary efficacy endpoint for Study 004 is the change from baseline in the Modified Neurological Impairment Score +7 (mNIS+7, Suanprasert et al., 2014) at 18 months.

The mNIS+7 is a 304-point composite measure of neurologic impairment that includes the following measures and components. Note that higher scores indicate greater impairment.

- Physical exam of lower limbs, upper limbs and cranial nerves in order to assess motor strength/weakness and determine the following component scores:

- o NIS-weakness (NIS-W)
- o NIS-reflexes (NIS-R)

- Electrophysiologic measures of small and large nerve fiber function in order to determine the sum of 5 NCS component scores that included assessment of the ulnar CMAP, ulnar SNAP, sural SNAP, tibial CMAP, and peroneal CMAP

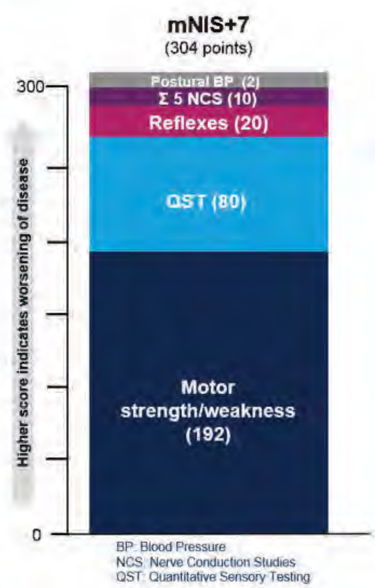
- Sensory testing to determine the QST score included assessing touch pressure by body surface area (QST-BSATP) and heat pain by body surface area (QST-BSAHP)

- Postural blood pressure was measured to assess autonomic function.

The following figure, copied from the submission, describes the components of the mNIS+7.

Figure 1: Modified Neurological Impairment Score + 7 (mNIS+7). Source: Applicant Submission

mNIS+7: Components, Scoring and Methodology		
Component	Body regions/nerves evaluated	Methodology including Scoring
Motor strength/weakness Neurological impairment score–weakness [NIS-W]) (192 points)	48 muscle group in lower limb, upper limb and body and cranial nerve components (Refer to 273 Appendix 1) Assessments are performed separately for the right and left side of the body	Physical exam evaluating motor strength is performed. A score of 0 (normal), 1 (25% weak), 2 (50% weak), 3 (75% weak) to 4 (paralysis) is applied to muscle groups. The scores for individual muscle groups are summated to get the total NIS-W score
Quantitative sensory testing (QST) (80 points)	Up to 10 anatomical sites (Refer to 273 Appendix 2) Assessments are performed on one side of the body	Touch pressure by body surface area (QST-BSA_{TP}) and heat pain by body surface area (QST-BSA_{HP}) are assessed at up to 10 anatomical sites using CASE (Computer Aided Sensory Evaluator) IV. A score of 0 (< 95th percentile), 1 (>=95th and <99th percentile) or 2 (>= 99th percentile) is applied at each anatomical site, summated across all anatomical sites and then multiplied by 2 (for 2 sides of body).
Reflexes (NIS-reflexes or NIS-R) (20 points)	5 reflexes in lower limb, upper limb and body (Refer to 273 Appendix 1). Assessments are performed separately for the right- and left-hand side of the body	A physical exam evaluating 10 reflexes is performed. A score of 0 (normal), 1 (decreased) or 2 (absent) is applied to each reflex and summated to get the total NIS-R score.
∑5 Nerve Conduction Studies (∑5 NCS) (10 points)	Ulnar CMAP Ulnar SNAP Sural SNAP Tibial CMAP Peroneal CMAP (Refer to 273 Appendix 1)	Nerve conduction studies are performed on one side of the body. Values are transformed to percentile value correcting for applicable variables of age, gender, height, or weight based on earlier studies of a large healthy subject reference cohort and expressed as points (ie, > 5th percentile= 0 points; ≤ 5th percentile – > 1st= 1 point and ≤ 1st percentile= 2 points).
Postural blood pressure (2 points)	autonomic nerves	Postural blood pressure is performed and points assigned based on the change in blood pressure with standing (blood pressure decrease of <20 mmHg = 0 points, blood pressure decrease of 20 to <30 mmHg = 1 point, blood pressure decrease ≥30 mmHg = 2 points
Abbreviations: CMAP=compound muscle action potential; mNIS+7=Modified Neurological Impairment Score +7; SNAP=sensory nerve action potential; VDT=vibration detection threshold		



Reviewer Comment:
The primary endpoint, mNIS+7, is composed of a clinical exam-based neuropathy impairment score (NIS) combined with electrophysiologic measures of small and large nerve fiber function (+7) such as nerve conduction studies (NCS), quantitative sensory testing (QST), and measurement of autonomic function (postural blood pressure). Many of the individual components of the score, such as nerve conduction studies, are clearly biomarkers that do not,

of themselves, represent direct clinical benefit. Other components of the score, such as motor and sensory function by neurological exam, also are not direct measures of clinical benefit, as differences detected by the physician might not be perceptible to the patient or result in improved function in daily activities. The mNIS+7 is an acceptable endpoint, but the results should be considered in the context of the results of the secondary endpoints, particularly the Norfolk QOL-DN.

Secondary Efficacy Endpoints

The study evaluated the difference between the study arms in the change from baseline in the following secondary efficacy outcome measures at 18 months:

- Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) questionnaire;
- Neurological impairment score (NIS)-weakness (NIS-W) score;
- Rasch-built Overall Disability Scale (R-ODS) score;
- Timed 10-meter walk test (10-MWT, gait speed);
- Modified body mass index (mBMI);
- Autonomic symptoms questionnaire (Composite Autonomic Symptom Score [COMPASS 31]).

<u>Norfolk QoL-DN</u>

The Norfolk QoL-DN questionnaire is a standardized 35-item patient-reported outcomes measure that is sensitive to the different features of diabetic neuropathy - small fiber, large fiber, and autonomic nerve function. There are 35 questions divided into 5 domains. The range of possible total scores is -4 to 136. Higher scores indicate a worse quality of life. A negative score is possible because in Question 31, “Very Good” is scored as –1, and “Excellent” is scored as –2. In Question 32, “Somewhat better” is scored as –1 and “Much better” is scored as -2.

Reviewer Comment: The Norfolk QoL-DN, evaluated in Vinik et al., 2005 and 2014, is a clinically meaningful endpoint that is appropriate for use in this study.
The remaining secondary and exploratory endpoints described below are acceptable.

<u>Ten-meter Walk Test</u>

Ability to ambulate (gait speed) was assessed through the 10-meter walk test (10-MWT). The walk had to be completed without assistance from another person; ambulatory aids such as canes and walkers were permitted.

<u>Rasch-built Overall Disability Scale</u>

An assessment of the disability each patient experienced was measured using the R-ODS. The R-ODS is comprised of a 24-item linearly weighted scale that captures activity and social participation limitations in patients.

Modified Body Mass Index

The nutritional status of patients was evaluated using the mBMI; calculated as the product of body mass index (BMI) (weight in kilograms divided by the square of height in meters) and serum albumin (g/L).

Patient-Reported Autonomic Neuropathy Symptoms (COMPASS 31)

To evaluate changes in autonomic symptoms, patients completed the COMPASS 31 questionnaire, consisting of 31 clinically selected questions. The questions evaluated 6 autonomic domains (orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor).

Exploratory Endpoints

The trial also evaluated the difference between treatment arms in the following exploratory endpoints at 18 months.

Neurological Impairment Score + 7 (NIS+7)
The NIS+7 is another composite neurological impairment score with a maximum of 270 points that differs from mNIS+7 in that it uses the full NIS (including NIS-sensation [NIS-S]), does not include QST, and has a +7 comprised of Σ5 NCS (sural SNAP, tibial motor distal latency, peroneal CMAP, peroneal motor nerve conduction velocity, and peroneal motor nerve distal latency), VDT, and Heart Rate Response to Deep Breathing (HRdb) described below.

Vibration Detection Threshold
Large nerve fiber function was further evaluated by VDT using the CASE IV device.

Heart Rate Response to Deep Breathing
The HRdb test evaluated small nerve fiber autonomic function by the cardio-vagal response. The average heart rate difference while taking eight deep breaths was measured using a Computer Aided Sensory Evaluator (CASE) device.

Large and small nerve fiber function
The large fiber nerve function was the sum of the point scores of the following: quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), nerve conduction studies sum of 5 attributes (NCS Σ5: ulnar CMAP, ulnar SNAP, sural SNAP,

tibial CMAP, and peroneal CMAP), vibration detection threshold (VDT).

The small fiber nerve function score was the sum of the point score of the following: QST HP by body surface area (QST-BSAHP), heart rate variability to deep breathing (HRdb), and postural blood pressure (BP) test.

Grip strength

Hand grip strength was measured by dynamometer. At each time point, 2 independent assessments (each assessment in triplicate) were performed.

EuroQOL (EQ-5D) questionnaire

The EQ-5D includes the EQ-5D-5L and the EQ visual analogue scale (EQ-VAS). Quality of life was assessed through the use of the EQ-5D-5L, a standardized 5 question instrument used as a measure of health outcomes. Overall health was assessed by the EQ-VAS.

Pathologic evaluation

Sensory and autonomic innervation was assessed through voluntary skin punch biopsies and analysis of intraepidermal nerve fiber density (IENFD), sweat gland nerve fiber density (SGNFD), and dermal amyloid content. Biopsies were read in a masked manner by a central laboratory.

Assessment of ambulation

Ambulation was assessed through the Familial Amyloidotic Polyneuropathy (FAP) stage and the Polyneuropathy Disability (PND) score.

Table 4: Polyneuropathy Disability Scores. Source: Study 004 CSR, p. 54

Stage	Description
0	No symptoms
I	Sensory disturbances but preserved walking capability
II	Impaired walking capacity but ability to walk without a stick or crutches
IIIA IIIB	Walking with the help of one stick or crutch Walking with the help of two sticks or crutches
IV	Confined to a wheelchair or bedridden

Table 5: Familial Amyloidotic Polyneuropathy Stages. Source: Study 004 CSR, p. 54

Stage	Description
0	No symptoms
I	Unimpaired ambulation; mostly mild sensory, motor, and autonomic neuropathy in the lower limbs
II	Assistance with ambulation required, mostly moderate impairment progression to the lower limbs, upper limbs, and trunk
III	Wheelchair-bound or bedridden; severe sensory, motor, and autonomic involvement of all limbs

Cardiac assessment
Cardiac function was assessed through echocardiogram, troponin I, and N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) levels. Echocardiograms were analyzed centrally. Quantification of cardiac biomarkers was performed at a central laboratory.

Pharmacodynamic (PD) biomarkers
Biomarkers that were assessed included TTR, retinol binding protein (RBP), and vitamin A.

Rapid disease progression
The proportion of patients was assessed in the patisiran-LNP and placebo groups who met the pre-defined criterion for rapid disease progression (defined as ≥24-point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline) at 9 months.

MRI neurography
Lower limb nerve injury was evaluated via voluntary magnetic resonance (MR) neurography approximately every 6 months in patients receiving either patisiran-LNP or placebo from France and Germany.

Safety Endpoints

Safety was assessed throughout the study by collecting AEs; clinical laboratory tests, including hematology, clinical chemistry (including liver function tests), thyroid function parameters, and

urinalysis; electrocardiograms; vital signs; physical examination findings; ophthalmology examinations, and the Columbia-Suicide Severity Rating Scale (C-SSRS).

Blood samples for clinical laboratory testing were collected prior to study drug dosing. Samples were sent to a central laboratory for analysis. The following table, copied from the submission, lists the clinical laboratory measurements.

Table 6: Clinical Laboratory Tests in Study 004. Source: CSR, p. 56

Hematology	
• Hematocrit • Hemoglobin • Red blood cell (RBC) count • White blood cell (WBC) count • Mean corpuscular volume • Mean corpuscular hemoglobin • Mean corpuscular hemoglobin concentration	• Neutrophils, absolute and % • Lymphocytes, absolute and % • Monocytes, absolute and % • Eosinophils, absolute and % • Basophils, absolute and % • Platelet count
Serum Chemistries	
• Aspartate transaminase (AST) • Alanine transaminase (ALT) • Sodium • Potassium • Blood urea nitrogen (BUN) • Creatinine	• Alkaline phosphatase • Bilirubin (total and direct) • Glucose • Phosphate • Albumin • Calcium
Coagulation Studies	
• Prothrombin time • Activated partial thromboplastin time (aPTT)	• International Normalized Ratio (INR)
Thyroid Function Tests	
• Thyroid stimulating hormone (TSH) • Thyroxine (Free T4)	• Triiodothyronine (Free T3)
Antidrug Antibodies	
• Anti-PEG antibodies	
Urinalysis	
• Visual inspection for color and appearance • pH • Specific gravity • Ketones • Protein • Glucose	• Leukocytes • Bilirubin • Nitrite • Urobilinogen • Microscopic inspection of sediment
Serology (viral loads for Hepatitis C were also assessed for eligibility of Hepatitis C positive patients)	
• Hepatitis B surface antibody (HbsAb) • Hepatitis B surface antigen (HbsAg)	• Anti–hepatitis C virus antibody (anti-HCVAb)
Cardiac Biomarkers	
• N-terminal prohormone of B-type natriuretic peptide (NT-proBNP)	• Troponin I
Other	
• β-human chorionic gonadotropin (women of child-bearing potential only; urine- or serum-based test)	
• Vitamin B12	
• Paraprotein by immunofixation electrophoresis (IFE)	

Pharmacokinetic Endpoints

Sparse blood samples were collected as outlined in the schedule of assessments for determination of the concentration of the patisiran drug substance (ALN-18328) and novel lipid excipients DLin-MC3-DMA and polyethylene glycol (PEG2000-C-DMG), which have not been used in other commercial products.

Spot urine samples for determination of ALN-18328 and 4-dimethylaminobutyric acid (the primary metabolite of DLin-MC3-DMA) were also collected as outlined in the schedule of assessments.

Immunogenicity Endpoint

Blood samples were collected to evaluate for the presence of antidrug antibodies (ADA) as outlined in the schedule of assessments. The presence of antidrug antibodies (defined as serum immunoglobulin (Ig) G (IgG)/IgM antibodies specific to PEG2000-C-DMG) was assessed. A validated ELISA method was used for the screening and confirmatory ADA assays. Serum samples were first analyzed with a screening assay. Samples testing ADA positive in the screening assay were further evaluated in a confirmatory assay. For the ADA samples that tested positive for ADA in the confirmatory assay, titer (expression of level of ADA) was then determined as the reciprocal of the highest dilution of the sample that yielded a positive result.

Reviewer Comment: The above safety endpoints are acceptable.

Statistical Analysis Plan

Reviewer Comment: Please refer to the statistical review for detailed evaluation of the applicant's planned statistical analysis.

The approach to multiple comparisons used by the applicant is as follows.

> Type I error rate for secondary endpoints was controlled by a hierarchical ordering procedure. Endpoints were tested in the following pre-specified hierarchy:
>
> 1. Norfolk QOL-DN questionnaire [Total Score]
> 2. NIS-W score
> 3. R-ODS
> 4. 10-meter walk test speed
> 5. mBMI
> 6. COMPASS-31 total score
>
> Only if a comparison was significant at a 2-sided 0.05 significance level, the next endpoint in the hierarchy would be formally tested; if a given comparison was not significant at a 2-sided 0.05 significance level, the subsequent tests would be performed and the results summarized, but statistical significance would not be inferred.

Populations for the analyses

The following patient populations were evaluated.

- Modified Intent-to-Treat (mITT) population: All patients who were randomized and received at least 1 dose of patisiran or placebo.
- Per-protocol (PP) population: All randomized patients who received at least 1 dose of patisiran or placebo, completed baseline and either 9-month or 18-month mNIS+7 and Norfolk QOL assessments, and did not experience any major protocol deviations.
- Safety population: All patients who received at least 1 dose of patisiran or placebo. For the safety analyses, patients were categorized on the basis of the test drug that was actually received.
- PK population: All patients in the Safety Population who provided at least 1 PK concentration measurement.

The primary population for efficacy analysis was the mITT population; the primary endpoint and the first secondary endpoint (Norfolk QOL) were analyzed using the PP population. The remaining secondary and exploratory efficacy endpoints were analyzed using the mITT population. Safety analysis was conducted in the safety population. PK analysis was conducted in the PK population.

Pre-specified methods of handling missing data

For the primary and secondary efficacy endpoints, the primary analysis was based on the mixed-effects model repeated measures (MMRM) method. Missing data were not imputed and were assumed to be missing-at-random (MAR).

For the primary endpoint mNIS+7 and the first secondary endpoint Norfolk QOL, sensitivity analyses were conducted to assess the impact of missing data.

Subgroup Analysis

Subgroup analyses were conducted to assess the consistency of treatment effect within subgroups with the following baseline characteristics:

- Age [≥65; <65 at randomization]
  - Sex [Male; Female]
  - Race [White; Non-White]
  - Region [North America; Western Europe; Rest of World]
  - NIS [< 50; ≥ 50]

- Genotype Class [Early-onset V30M; Other]
- Previous Tetramer Use [Yes; No]
- Genotype [V30M; non-V30M]
- FAP Stage [I; II & III]

Subgroup analyses were performed for the primary endpoint mNIS+7 and Norfolk QOL-DN using MMRM models with baseline mNIS+7 score as a continuous covariate and genotype (V30M vs. non-V30M) as a factor.

Protocol Amendments

The original protocol was finalized on 8/15/2013; one patient was enrolled under the original protocol. There were 5 amendments to the protocol; a majority of patients enrolled in the study under these protocol amendments.

See Appendix 13.12 for a table of all protocol amendments, copied from the submission.

Data Quality and Integrity: Sponsor's Assurance

The applicant used the following methods for assuring data quality and integrity, which are adequate.

The Investigator was accountable for the conduct of the trial. If any responsibilities were delegated, the Investigator maintained a list of appropriately qualified staff to whom trial related duties had been delegated. The Sponsor supplied electronic CRFs for each patient. The Investigator allowed designated Sponsor representatives and regulatory bodies to have direct access to the source documents to verify the data reported in the CRFs. Each completed CRF was reviewed and signed by the Investigator or designee. Twenty-one sites were audited for compliance with GCP requirements.

6.1.2. **Study 004 Results**

Compliance with Good Clinical Practices

All clinical studies were conducted in accordance with Good Clinical Practice and the Declaration of Helsinki and local requirements, and in consideration of applicable regulatory Guidance.

Financial Disclosure

The applicant has adequately disclosed financial interests/ arrangements with clinical investigators as recommended in the guidance for industry Financial Disclosure by Clinical

Investigators.

Patient Disposition

Patient disposition is described in the following table, copied from the applicant.
Note that discontinuations due to progressive disease, adverse events, and death were higher in the placebo group (5.2%, 9.1%, 5.2%, respectively) than in the patisiran group (0.7%, 2.0%, 3.4%, respectively). Withdrawals from the study due to adverse events and death were also higher in the placebo group. *These observations are supportive of the safety of patisiran.*

Table 7: Patient Disposition. Source: Study 004 CSR, p. 80

Disposition	**Placebo (N=77)**	**Patisiran-LNP (N=148)**	**Overall (N=225)**
Total number of patients	**N (%)**		
Randomized	77	148	225
Treated	77 (100.0)	148 (100.0)	225 (100.0)
Completed treatment[a]	48 (62.3)	137 (92.6)	185 (82.2)
Completed study[b]	55 (71.4)	138 (93.2)	193 (85.8)
Discontinuation of treatment	29 (37.7)	11 (7.4)	40 (17.8)
Primary reason for treatment discontinuation			
Adverse event	7 (9.1)	3 (2.0)	10 (4.4)
Death	4 (5.2)	5 (3.4)	9 (4.0)
Progressive Disease [c]	4 (5.2)	1 (0.7)	5 (2.2)
Physician Decision	2 (2.6)	0	2 (0.9)
Protocol deviation	0	1 (0.7)	1 (0.4)
Withdrawal by subject	12 (15.6)	1 (0.7)	13 (5.8)
Withdrawal from study	22 (28.6)	10 (6.8)	32 (14.2)
Primary reason for study withdrawal			
Adverse Event	6 (7.8)	2 (1.4)	8 (3.6)
Death	4 (5.2)	6 (4.1)	10 (4.4)
Physician decision	1 (1.3)	0	1 (0.4)
Protocol deviation	0	1 (0.7)	1 (0.4)
Withdrawal by subject	11 (14.3)	1 (0.7)	12 (5.3)
Patients with rapid disease progression[d]	6 (7.8)	1 (0.7)	7 (3.1)

Disposition	Placebo (N=77)	Patisiran-LNP (N=148)	Overall (N=225)
Patients who discontinued treatment but completed study	8 (10.4)	1 (0.7)	9 (4.0)
Patients who completed treatment but withdrew from study	1 (1.3)	0	1 (0.4)

Abbreviations: CRF=case report form, FAP=Familial amyloidotic polyneuropathy; LNP=Lipid nanoparticle.
Note: Percentages for populations, discontinuation, and reasons for discontinuation are based on the number randomized.
[a] A patient was considered to have completed study treatment if they had completed the drug regimen without permanently stopping treatment prior to the last dose at the Week 78 visit. Patient completion is indicated by the Investigator on the End of Treatment CRF.
[b] A patient was considered to have completed the study if they completed protocol-specified procedures through the Month 18 efficacy assessment visit (Week 79-80). Patient completion is indicated by the Investigator on the End of Study CRF.
[c] Patients with rapid disease progression who decided to stop treatment due to this progressive disease.
[d] Rapid disease progression is defined as patients with a ≥24 point increase from baseline in mNIS+7 and a ≥ 1 level increase from baseline in FAP stage at Month 9 as determined by the Clinical Adjudication Committee.

Protocol Violations/Deviations

A summary of major protocol deviations is in the following table, copied from the applicant. One patient ((b) (6)) in the patisiran-LNP group discontinued treatment due to a protocol deviation (elevated bilirubin levels at baseline); there were no protocol deviations leading to study discontinuation in the placebo group.

These deviations do not compromise the efficacy and safety results of the study.

Table 8: List of Major Protocol Deviations

Patient ID	Treatment Group	Protocol Deviation Criteria	Summary	Excluded from PP Population
(b) (6) [redacted]	Patisiran-LNP	Inclusion criteria	Bilirubin level was above the limit of normal at baseline (bilirubin was 19 µmol/L when ULN was 18.8 µmol/L). The liver function test results were available to the Investigator after the patient had been randomized and had received the first dose of study drug. Liver function test results were subsequently re-assessed showing similar levels of bilirubin (20.9 µmol/L) at baseline (Appendix 16.2.8.3). The patient was withdrawn from the study due to this protocol deviation after having received 1 dose of study drug	No
	Patisiran-LNP	Exclusion	vitamin B12 below the lower limit of normal at screening	Yes
	Patisiran-LNP	Exclusion	vitamin B12 below the lower limit of normal at screening	Yes
	Patisiran-LNP	GCP including ICF	Initially this patient had not consented to have skin punch biopsies collected, yet samples were collected without refusal from the patient (Appendix 16.2.2.3.2). The patient subsequently signed a form agreeing to have skin punch biopsies collected and assessed for this study. This patient completed study drug and	No

Patient ID	Treatment Group	Protocol Deviation Criteria	Summary	Excluded from PP Population
			the study (Appendix 16.2.1.1).	
(b) (6) [redacted]	Patisiran-LNP	Potential unblinding	Site clinical research coordinator (CRC) was exposed to unblinded information on a memo from the pharmacist with lot numbers. The site agreed to remove the CRC and retain her on the unblinded team; however, per the delegation log, prior responsibilities had not changed, including checking vital signs, ECG. This CRC was not associated with efficacy assessments (source is in the TMF).	No
	Placebo	Exclusion	The patient had presented with monoclonal gammopathy (Appendix 16.2.4.5.2) that was considered as mild by the Investigator and not the main origin of neuropathy, and therefore proceeded to randomize the patient into the study. The patient received study drug until Day 127 (Appendix 16.2.1.1). The patient was discontinued from treatment due to an AE of peripheral arterial occlusive disease of severe intensity that was considered unlikely related to study drug. The patient was subsequently withdrawn from the study on Day 327 (Appendix 16.2.1.1 and Appendix 16.2.7.1).	Yes

Abbreviations: AE=adverse event; CRC=clinical research coordinator; ECG=electrocardiogram; TMF=Trial Master File; ULN=upper limit of normal.

Table of Demographic Characteristics

Study patient demographics are described in the following tables, based on the study data, and in Section 8.2.2. Study 004 enrolled 225 patients. The mean age was 61 years with a median age of 62 years (range 24 to 83 years). 74% of patients were male, 72% were White/Caucasian, and 23% were Asian. Patients were from North America (21%), Western Europe (44%), and rest of world (ROW) (36%). *Overall, there appears to be an acceptable balance of demographic characteristics between the control and treatment groups that adequately represents the demographics of the intended patient population. Note that the different genotypes of hATTR amyloidosis can have different prevalence in males and females, which may account for the gender imbalance in the study population.*

Table 9: Demographics of Study 004. Source: Submitted data

Subject Population by Treatment

Actual Treatment for Period 01	Count	% of Total
Patisiran 0.3 mg/kg	148	65.8%
PLACEBO	77	34.2%
All	225	100.0%

Subject Population by Age

		Actual Treatment for Period 01		
		Patisiran 0.3 mg/kg	PLACEBO	All
Age	N	148	77	225
	Mean	59.58	62.17	60.47
	Std Dev	11.96	10.76	11.61
	Min	24	34	24
	Quantiles25	53	57	54
	Median	62	63	62
	Quantiles75	68	72	69
	Max	83	80	83

Subject Population by Sex

	Actual Treatment for Period 01					
	Patisiran 0.3 mg/kg		PLACEBO			
Sex	Count	Column %	Count	Column %	Count	% of Total
F	39	26.4%	19	24.7%	58	25.78%
M	109	73.6%	58	75.3%	167	74.22%
All	148	100.0%	77	100.0%	225	100.00%

Subject Population by Race

	Actual Treatment for Period 01					
	Patisiran 0.3 mg/kg		PLACEBO			
Race	Count	Column %	Count	Column %	Count	% of Total
ASIAN	27	18.2%	25	32.5%	52	23.11%
BLACK OR AFRICAN AMERICAN	4	2.7%	1	1.3%	5	2.22%
MULTIPLE	2	1.4%	0	0.0%	2	0.89%
OTHER	1	0.7%	0	0.0%	1	0.44%
UNKNOWN	1	0.7%	1	1.3%	2	0.89%
WHITE	113	76.4%	50	64.9%	163	72.44%
All	148	100.0%	77	100.0%	225	100.00%

Subject Population by Ethnicity

	Actual Treatment for Period 01					
	Patisiran 0.3 mg/kg		PLACEBO			
Ethnicity	Count	Column %	Count	Column %	Count	% of Total
HISPANIC OR LATINO	17	11.5%	11	14.3%	28	12.44%
NOT HISPANIC OR LATINO	130	87.8%	65	84.4%	195	86.67%
UNKNOWN	1	0.7%	1	1.3%	2	0.89%

	Actual Treatment for Period 01					
	Patisiran 0.3 mg/kg		PLACEBO			
Ethnicity	Count	Column %	Count	Column %	Count	% of Total
All	148	100.0%	77	100.0%	225	100.00%

Subject Population by Study Site

		Actual Treatment for Period 01					
		Patisiran 0.3 mg/kg		PLACEBO			
Country	Study Site Identifier	Count	Column %	Count	Column %	Count	% of Total
ARG	100	1	0.7%	0	0.0%	1	0.44%
	All	1	0.7%	0	0.0%	1	0.44%
BGR	140	7	4.7%	1	1.3%	8	3.56%
	All	7	4.7%	1	1.3%	8	3.56%
BRA	040	1	0.7%	0	0.0%	1	0.44%
	041	0	0.0%	2	2.6%	2	0.89%
	All	1	0.7%	2	2.6%	3	1.33%
CAN	058	4	2.7%	1	1.3%	5	2.22%
	All	4	2.7%	1	1.3%	5	2.22%
CYP	180	2	1.4%	2	2.6%	4	1.78%
	All	2	1.4%	2	2.6%	4	1.78%
DEU	070	7	4.7%	1	1.3%	8	3.56%
	071	6	4.1%	1	1.3%	7	3.11%
	All	13	8.8%	2	2.6%	15	6.67%
ESP	060	4	2.7%	1	1.3%	5	2.22%
	061	2	1.4%	3	3.9%	5	2.22%
	062	0	0.0%	3	3.9%	3	1.33%
	063	1	0.7%	3	3.9%	4	1.78%
	All	7	4.7%	10	13.0%	17	7.56%
FRA	050	15	10.1%	7	9.1%	22	9.78%
	051	3	2.0%	2	2.6%	5	2.22%
	052	2	1.4%	1	1.3%	3	1.33%
	053	3	2.0%	2	2.6%	5	2.22%
	All	23	15.5%	12	15.6%	35	15.56%
GBR	170	1	0.7%	1	1.3%	2	0.89%
	All	1	0.7%	1	1.3%	2	0.89%
ITA	030	2	1.4%	0	0.0%	2	0.89%
	031	1	0.7%	0	0.0%	1	0.44%
	032	3	2.0%	2	2.6%	5	2.22%
	All	6	4.1%	2	2.6%	8	3.56%
JPN	160	6	4.1%	3	3.9%	9	4.00%
	161	0	0.0%	2	2.6%	2	0.89%
	163	1	0.7%	4	5.2%	5	2.22%
	All	7	4.7%	9	11.7%	16	7.11%
KOR	130	4	2.7%	1	1.3%	5	2.22%
	131	4	2.7%	1	1.3%	5	2.22%
	All	8	5.4%	2	2.6%	10	4.44%
MEX	110	11	7.4%	4	5.2%	15	6.67%

		Actual Treatment for Period 01					
		Patisiran 0.3 mg/kg		PLACEBO			
Country	**Study Site Identifier**	**Count**	**Column %**	**Count**	**Column %**	**Count**	**% of Total**
	All	11	7.4%	4	5.2%	15	6.67%
NLD	037	1	0.7%	1	1.3%	2	0.89%
	All	1	0.7%	1	1.3%	2	0.89%
PRT	020	2	1.4%	4	5.2%	6	2.67%
	021	4	2.7%	0	0.0%	4	1.78%
	All	6	4.1%	4	5.2%	10	4.44%
SWE	010	5	3.4%	4	5.2%	9	4.00%
	All	5	3.4%	4	5.2%	9	4.00%
TUR	150	4	2.7%	1	1.3%	5	2.22%
	All	4	2.7%	1	1.3%	5	2.22%
TWN	120	6	4.1%	6	7.8%	12	5.33%
	121	2	1.4%	4	5.2%	6	2.67%
	All	8	5.4%	10	13.0%	18	8.00%
USA	080	5	3.4%	1	1.3%	6	2.67%
	081	4	2.7%	2	2.6%	6	2.67%
	082	3	2.0%	2	2.6%	5	2.22%
	084	2	1.4%	1	1.3%	3	1.33%
	085	10	6.8%	2	2.6%	12	5.33%
	086	3	2.0%	0	0.0%	3	1.33%
	087	1	0.7%	0	0.0%	1	0.44%
	090	2	1.4%	0	0.0%	2	0.89%
	091	1	0.7%	0	0.0%	1	0.44%
	092	1	0.7%	0	0.0%	1	0.44%
	093	1	0.7%	1	1.3%	2	0.89%
	All	33	22.3%	9	11.7%	42	18.67%

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

Additional baseline characteristics of the study population are described in Section 8.2.2.

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

The applicant describes treatment compliance results as follows, indicating *similar rates of missed doses in the patisiran and placebo groups*.

A total of 100 (67.6%) and 51 (66.2%) patients in the patisiran-LNP and placebo groups, respectively, did not miss any doses between the first and last dose. A total of 6 (4.1%) and 3 (3.9%) patients in the patisiran-LNP and placebo groups, respectively, missed 3 or more doses. A total of 3 (2.0%) and 2 (2.6%) patients in the patisiran-LNP and placebo groups, respectively, missed 2 consecutive doses. There were no patients who missed 3 or more consecutive doses.

Concomitant medications taken by at least 15% of patients were retinol (patisiran-LNP 77.0%, placebo 72.7%), which was required by the protocol; paracetamol (patisiran-LNP 43.2%, placebo 35.1%), pregabalin (patisiran-LNP 30.4%, placebo 35.1%), gabapentin (patisiran-LNP 25.7%, placebo 29.9%), furosemide (patisiran-LNP 23.0%, placebo 37.7%), loperamide (patisiran-LNP 22.3%, placebo 18.2%), acetylsalicylic acid (patisiran-LNP 16.9%, placebo 16.9%), and omeprazole (patisiran-LNP 12.8%, placebo 15.6%). Twelve (8.1%) patisiran-LNP patients and 4 (5.2%) placebo patients did not have retinol or a vitamin A-containing supplement recorded as a concomitant medication. *Overall concomitant medication use was similar between the patisiran and placebo groups.*

There were no applicable rescue medications for this trial.

Efficacy Results – Primary Endpoint: Change from baseline in the Modified Neurological Impairment Score +7 (mNIS+7) at 18 months.

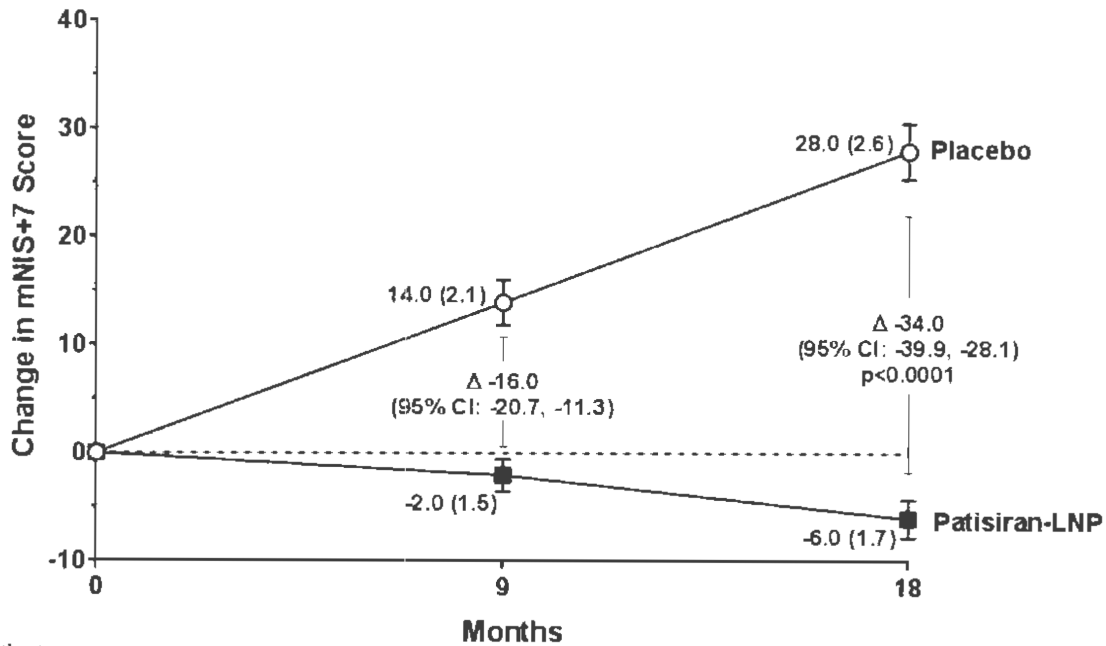
The table and figures below show the applicant's analysis of the primary endpoint. Note that higher mNIS+7 scores indicate greater impairment. There is a statistically significant difference in mNIS+7 scores between the patisiran and placebo groups, *indicating an improvement of neurological function in the patisiran group and a worsening in the placebo group.*

Table 10: Change from Baseline mNIS+7 Score at Month 18, Using MMRM Model (mITT Population)

Visit[a]	Actual/ Change	Statistic[b]	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Baseline	Actual	N	77	148
		Mean	74.61	80.93
		SD	37.041	41.507
		Median	71.50	76.94
		Min, Max	11.0, 153.5	8.0, 165.0
Month 18	Actual	N	51	137
		Mean	101.09	75.13
		SD	45.35	43.18
		Median	93.88	70.63
		Min, Max	21.5, 190.1	8.0, 198.9
	Change	N	51	137
		Mean	27.90	-4.19
		SEM	3.116	1.553
		Median	26.50	-4.00
		Min, Max	-15.1, 84.3	-49.5, 59.6
		LS Mean (SEM)	27.96 (2.602)	-6.03 (1.739)
		95% CI	22.83, 33.09	-9.46, -2.60
		LS Mean (SEM) Difference (Patisiran - Placebo)	-	**-33.99 (2.974)**
		95% CI	-	**-39.86, -28.13**
		p-value	-	**9.262E-24**

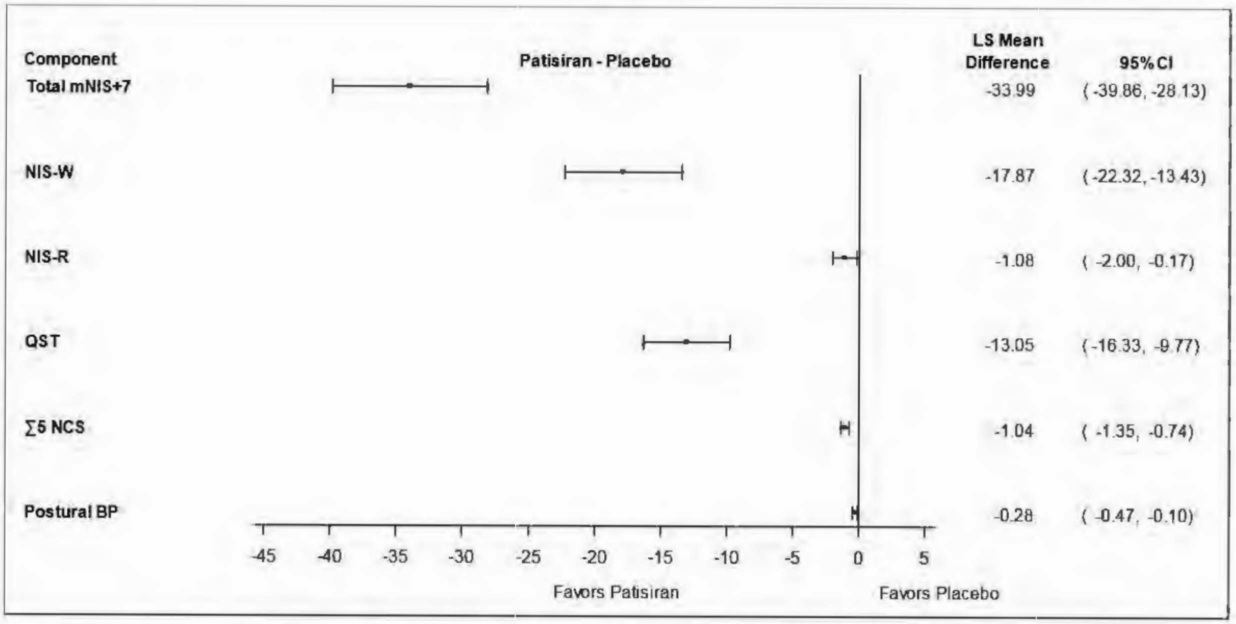
Abbreviations: CI=confidence interval; LNP=lipid nanoparticle; LS=least squares; max=maximum; min=minimum; mITT=modified intent-to-treat; MMRM=mixed-effect model repeated measures; mNIS + 7=Modified Neurologic Impairment Score + 7; SD=standard deviation; SEM=standard error of the mean.
Note: In the MMRM model, the outcome variable is change from baseline in mNIS + 7. The model includes baseline mNIS + 7 score as covariate and fixed-effect terms including treatment group, visit, treatment-by-visit interaction, genotype, age at hATTR symptom onset, previous tetramer stabilizer use, and region.
[a] Baseline and Month 18 are the averages of 2 assessments performed at least 24 hours but no more than 7 days apart.
[b] LS means, SEM, differences in LS means, 95% CIs, and Month 18 p-value from MMRM model.

Figure 2: Change in mNIS+7 at 9 and 18 Months, MMRM Analysis (mITT) of Study 004. Higher mNIS+7 scores indicate greater impairment. Source: Applicant submission.



N of patients			
Placebo	77	67	51
Patisiran-LNP	148	141	137

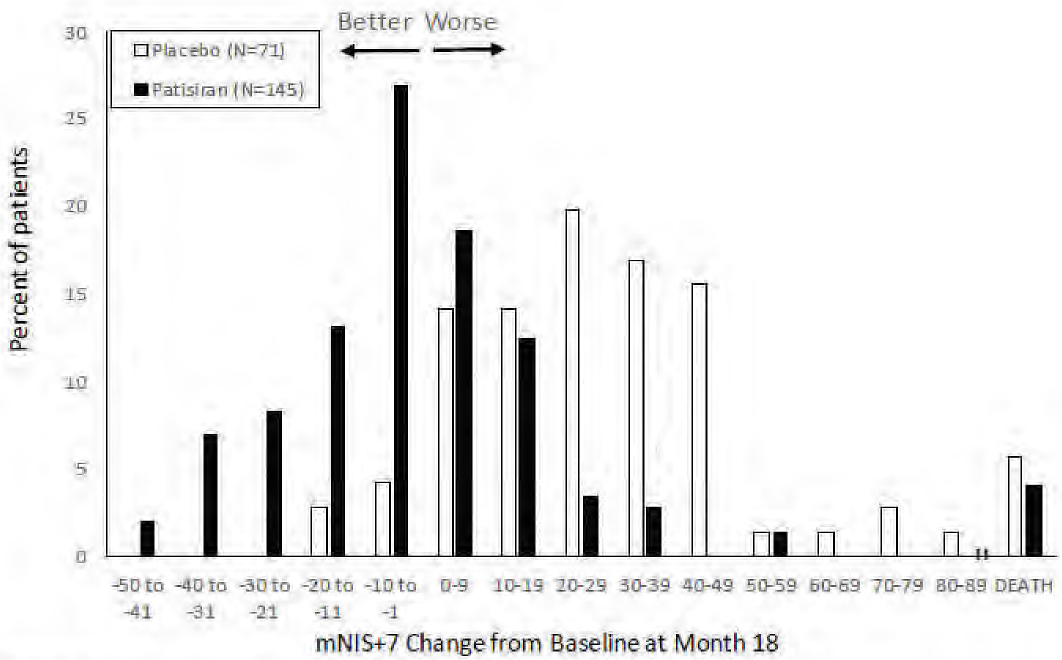
Figure 3: mNIS+7 Component Analysis, Change from Baseline at 18 Months, MMRM Analysis (mITT) of Study 004. Source: Applicant submission.



Abbreviations: mNIS+7, modified Neuropathy Impairment Score +7; NIS-W, Neuropathy Impairment Score – Weakness; NIS-R, Neuropathy Impairment Score – Reflex; QST, quantitative sensory testing; NCS, nerve conduction studies; BP, blood pressure

The following histogram for the primary endpoint was generated by the FDA statistician from the submitted data.

Figure 4: Primary Endpoint Histogram Comparing mNIS+7 Change from Baseline at Month 18 between Patisiran and Placebo Groups. Source: Analysis of submitted data for Study 004.



mNIS+7 change scores are rounded to the nearest whole number.
Last available scores were used.

Reviewer Comment: The observed improvement of neuropathy as evidenced by the reduction in mNIS+7 score is not consistent with the natural history of hereditary TTR amyloidosis polyneuropathy and supports the efficacy of patisiran. The effect on the mNIS+7 appears to be driven primarily by an effect on muscle strength and QST. Although strength testing can potentially be affected by subject motivation in cases of unblinding, motivation would be unlikely to have an effect on QST. In addition, the stability of benefit in many patients over the course of the 18-month trial is highly inconsistent with the known disease progression. It would be very unlikely that this finding could be the result of any theoretical bias. These results have been independently verified by the Agency biometrics reviewer for this application.

Data Quality and Integrity

No clinical research sites were identified by OSI as potentially fraudulent. There were delays in reporting some serious adverse events, but there were no omissions. Approximately 11% of subjects in Germany and Italy had a birthdate of January 1, suggesting that the actual month and day may have been unknown or that subjects declined to give their actual birthdate. Birth years did not appear to be affected. There is no detectable data duplication in any other

variables. This finding does not affect efficacy or safety evaluations.

Efficacy Results – Secondary endpoints evaluated at 18 months

As seen in the following table copied from the submission, all secondary efficacy endpoints had statistically significant results supporting the efficacy of patisiran. As previously noted, the analysis of these endpoints was appropriately controlled for multiple comparisons. Individual secondary endpoint results are discussed further below. The applicant's verbatim descriptions of the secondary efficacy outcome measures are provided below, and are confirmed to be accurate by this reviewer. The results of the analyses of the secondary efficacy measures have been independently verified by the biometrics reviewer for this application.

Table 11: Summary of Secondary Endpoint Analyses, MMRM Analysis (mITT). Source: Applicant submission for Study 004

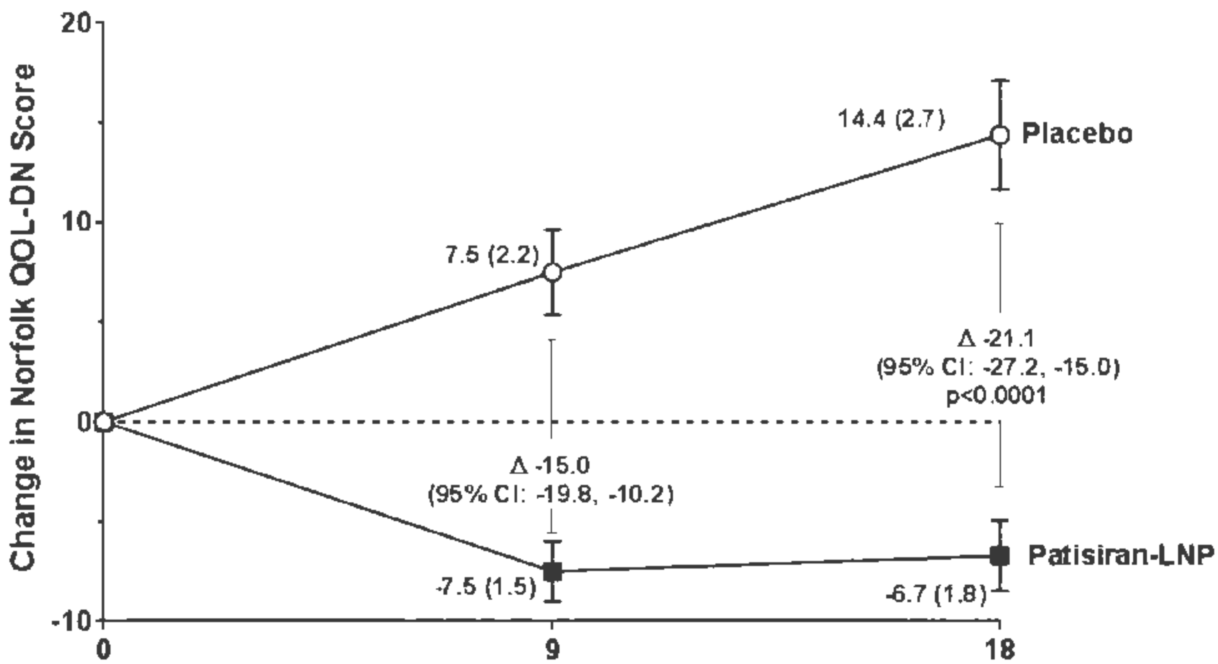
Secondary Endpoint	Baseline, Mean (SD)		Change from Baseline at 18 months, LS Mean (SEM)		Treatment Difference, LS Mean (95% CI) (Patisiran-LNP – Placebo)	p-value
	Patisiran-LNP N=148	Placebo N=77	Patisiran-LNP	Placebo		
Norfolk QoL-DN[a]	59.6 (28.2)	55.5 (24.3)	-6.7 (1.8)	14.4 (2.7)	-21.1 (-27.2, -15.0)	<0.0001
NIS-W[a]	32.7 (25.2)	29.0 (23.0)	0.05 (1.3)	17.9 (2.0)	-17.9 (-22.3, -13.4)	<0.0001
R-ODS[b]	29.7 (11.5)	29.8 (10.8)	0.0 (0.6)	-8.9 (0.9)	9.0 (7.0, 10.9)	<0.0001
10-meter walk test (m/sec)[b]	0.80 (0.40)	0.79 (0.32)	0.08 (0.02)	-0.24 (0.04)	0.31 (0.23, 0.39)	<0.0001
mBMI[c]	970 (211)	990 (214)	-3.7 (9.6)	-119 (14.5)	116 (82, 149)	<0.0001
COMPASS-31[a]	30.6 (17.6)	30.3 (16.4)	-5.3 (1.3)	2.2 (1.9)	-7.5 (-11.9, -3.2)	0.0008

Abbreviations: QOL-DN, Quality of Life – Diabetic Neuropathy; NIS-W, Neuropathy Impairment Score – Weakness; R-ODS, Rasch-built Overall Disability Scale; mBMI, modified body mass index; COMPASS – Composite Autonomic Symptom Scale
All endpoints analyzed using the mixed effect model repeated measures method (MMRM).
[a]A lower number indicates less impairment/less disability/fewer symptoms
[b]A higher number indicates less impairment
[c]mBMI: BMI (kg/m^2) multiplied by serum albumin (g/L); a higher number indicates better nutritional status

Norfolk QoL-DN change from baseline over time

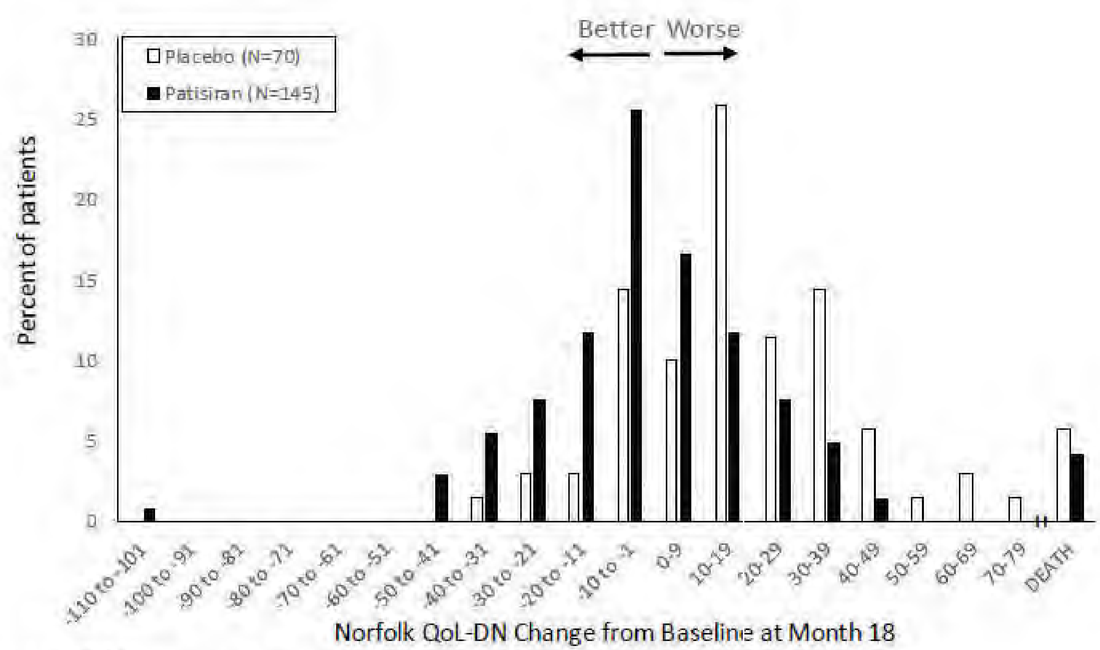
As shown in the following table copied from the submission, there was an improvement in the Norfolk quality of life score in the patisiran group, compared to a worsening in the placebo group.

Figure 5: Change in Norfolk QOL-DN at 9 and 18 Months, MMRM Analysis (mITT) of Study 004. Source: Applicant submission.



The following histogram for the Norfolk QOL-DN secondary endpoint was generated by the FDA statistician from the submitted data.

Figure 6: Histogram Comparing Norfolk QOL-DN Change from Baseline at Month 18 between Patisiran and Placebo Groups. Source: FDA statistician's analysis of submitted data for Study 004.



Last available scores were used.

Reviewer Comment: The observed improvement of quality of life as evidenced by the Norfolk QOL-DN score supports the efficacy of patisiran.

Neurological impairment score (NIS)-weakness (NIS-W) score

As shown in the following table copied from the submission, there was less worsening in the NIS-W score, a measure of muscle strength, in the patisiran group compared to the placebo group. The total possible NIS-W score is 192 points. A decrease from baseline in NIS-W score represents improvement, and an increase from baseline represents worsening. *This result is*

not independent of the primary endpoint, as the NIS-W is a component of the mNIS+7.

Table 12: NIS-W Change from Baseline Over Time, MMRM Model (mITT Population). Source: Study 004 CSR, p. 118.

Visit[a]	Actual/ Change	Statistic[b]	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Baseline	Actual	N	77	148
		Mean	29.03	32.69
		SD	22.950	25.226
		Median	27.50	29.50
		Min, Max	0.0, 79.5	0.0, 103.6
Month 18	Actual	N	51	137
		Mean	46.32	33.72
		SD	31.77	28.34
		Median	45.88	28.00
		Min, Max	0.0, 99.3	0.0, 119.4
	Change	N	51	137
		Mean	19.53	1.94
		SEM	2.473	1.120
		Median	13.25	0.00
		Min, Max	-1.0, 63.4	-31.5, 47.6
		LS Mean (SEM)	17.93 (1.959)	0.05 (1.306)
		95% CI	14.07, 21.79	-2.52, 2.63
		LS Mean (SEM) Difference (Patisiran - Placebo)	-	**-17.87 (2.254)**
		95% CI	-	**-22.32, -13.43**
		p-value	-	**1.404E-13**

Abbreviations: CI=confidence interval; FAP= familial amyloidotic polyneuropathy; LNP=lipid nanoparticle; LS= least squares; hATTR=hereditary transthyretin-mediated; mITT=modified intent-to-treat; max=maximum; min=minimum; MMRM=mixed-effect model repeated measures; NIS=Neurologic Impairment Score; NIS-W=NIS-weakness; QoL-DN=Quality of Life-Diabetic Neuropathy; SD=standard deviation; SEM=standard error of the mean.
In the MMRM model, the outcome variable is change from baseline in NIS-W. The model includes baseline NIS-W score as covariate and fixed effect terms including treatment group, visit, treatment-by-visit interaction, genotype, age at hATTR symptom onset, previous tetramer stabilizer use and region
Notes: Data collected post alternative treatment are excluded from analysis.
[a] Baseline, Month 9, and Month 18 are the averages of 2 assessments performed at least 24 hours but no more than 7 days apart.
[b] LS means, SEM, differences in LS means, 95% CIs and Month 18 p-value from MMRM model.

Rasch-built Overall Disability Scale (R-ODS) score change from baseline over time

The R-ODS is a patient-reported measure of level of disability on a scale of 0-48, with 0 being

the worst and 48 the best. As seen in the following table, copied from the submission, there was worsening in the placebo group and no change (LS mean change from baseline) in the patisiran group at 18 months. *This result is consistent with the positive findings of the primary endpoint.*

Table 13: Analysis of Mean Change from Baseline at 18 months in R-ODS, MMRM Model (mITT Population). Source: Study 004 CSR, p. 119

Visit	Actual/ Change	Statistic[b]	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Baseline[a]	Actual	N	76	148
		Mean	29.8	29.7
		SD	10.76	11.51
		Median	30.5	29.5
		Min, Max	3, 48	2, 48
Month 18	Actual	N	54	138
		Mean	21.0	29.5
		SD	13.36	12.70
		Median	19.5	31.0
		Min, Max	2, 48	1, 48
	Change	N	54	138
		Mean	-9.8	-0.8
		SEM	1.01	0.57
		Median	-9.5	-1.0
		Min, Max	-23, 6	-20, 27
		LS Mean (SEM)	-8.9 (0.88)	0.0 (0.59)
		95% CI	-10.7, -7.2	-1.1, 1.2
		LS Mean (SEM) Difference (Patisiran - Placebo)	-	**9.0 (1.01)**
		95% CI	-	**7.0, 10.9**
		p-value	-	**4.066E-16**

Abbreviations: Abbreviations: CI=confidence interval; FAP= familial amyloidotic polyneuropathy; LNP=lipid nanoparticle; LS= least squares; mITT=modified intent-to-treat; max=maximum; min=minimum; MMRM=mixed-effect model repeated measures; R-ODS=Rausch-built Overall Disability Scale; SD=standard deviation; SEM=standard error of the mean.
Notes: In the MMRM model, the outcome variable is change from baseline in R-ODS value. The model includes baseline score as covariate and fixed effect terms including treatment group, visit, treatment-by-visit interaction, baseline NIS, genotype, age at hATTR symptom onset, previous tetramer stabilizer use and region.
[a] Baseline is defined as the measurement closest to and prior to the first dose of study drug.
[b] LS means, SEM, differences in LS means, 95% CIs, and Month 18 p-value from MMRM model.

Timed 10-meter walk test (10-MWT, gait speed) change from baseline over time

The 10-meter walk test (10-MWT) is a measure of walking ability and gait speed expressed as a

rate in meters/second . An increase in gait speed from baseline indicates improvement, and a decrease from baseline
indicates worsening. As seen in the following table, copied from the submission, there was a mean improvement in the patisiran group compared to mean worsening in the placebo group at 18 months. *This result is consistent with the positive finding of the primary endpoint.*

Table 14: 10-MWT (Gait Speed in meters per second) Change from Baseline over time, MMRM Model (mITT Population). Source: Study 004 CSR, p. 123.

Visit[a]	Actual/ Change	Statistic[b]	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Baseline	Actual	N	77	147
		Mean	0.790	0.795
		SD	0.3188	0.4009
		Median	0.800	0.765
		Min, Max	0.00, 1.53	0.06, 2.00
Month 18	Actual	N	55	138
		Mean	0.555	0.845
		SD	0.395	0.498
		Median	0.571	0.870
		Min, Max	0.00, 1.68	0.00, 2.18
	Change	N	55	138
		Mean	-0.260	0.040

Visit[a]	Actual/ Change	Statistic[b]	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
		SEM	0.0389	0.0225
		Median	-0.259	0.036
		Min, Max	-0.86, 0.50	-1.25, 1.33
		LS Mean (SEM)	-0.235 (0.0358)	0.077 (0.0242)
		95% CI	-0.305, -0.164	0.029, 0.124
		LS Mean (SEM) Difference (Patisiran - Placebo)		**0.311 (0.0415)**
		95% CI		**0.230, 0.393**
		p-value		**1.875E-12**

Abbreviations: CI=confidence interval; LS=least squares; max=maximum; min=minimum; mITT=modified intent-to-treat; MMRM=mixed-effect model repeated measures; 10-MWT=10 meter walk test; SD=standard deviation; SEM=standard error of the mean.
Note: the unit for 10-MWT is meter/second.
In the MMRM model, the outcome variable is change from baseline in 10-meter walk test result.
The model includes baseline test result as covariate and fixed effect terms including treatment group, visit, treatment-by-visit interaction, baseline NIS, genotype, age at hATTR symptom onset, previous tetramer stabilizer use and region.
[a] Baseline, Month 9, and Month 18 values are equal to 10 meters divided by the average time taken to complete the two assessments at each visit. The walk speed for patients unable to perform the walk is imputed as 0.
[b] LS Means, SEM, Differences in LS Means, 95% CIs and Month 18 p-value from MMRM model.

Modified body mass index (mBMI) change from baseline over time

The mBMI is the product of BMI multiplied by the concentration of serum albumin. An increase in mBMI from baseline suggests improvement in nutritional status, and a decrease from baseline suggests worsening of nutritional status. As seen in the following table, copied from the submission, there was less decrease in the mBMI in the patisiran group compared to the placebo group. *This result is consistent with the positive finding of the primary endpoint.*

Table 15: Modified BMI Change from Baseline at Month 18, MMRM Model (mITT Population). Source: Study 004 CSR, p. 125).

Visit	Actual/ Change	Statistic[b]	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Baseline[a]	Actual	N	77	148
		Mean	989.9	969.7
		SD	214.19	210.45
		Median	959.7	971.3
		Min, Max	569, 1508	522, 1428
Month 18 (Day 546)	Actual	N	52	133
		Mean	892.7	975.4
		SD	221.10	228.56
		Median	869.8	983.4
		Min, Max	493, 1382	412, 1662
	Change	N	52	133
		Mean	-122.1	-6.5
		SEM	13.41	9.70
		Median	-114.5	-8.4
		Min, Max	-393, 86	-307, 341
		LS Mean (SEM)	-119.4 (14.51)	-3.7 (9.57)

Visit	Actual/ Change	Statistic[b]	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
		95% CI	-148.0, -90.8	-22.6, 15.1
		LS Mean (SEM) Difference (Patisiran - Placebo)	-	**115.7 (16.91)**
		95% CI	-	**82.4, 149.0**
		p-value	-	**8.832E-11**

Abbreviations: BMI=body mass index; CI=confidence interval; LS= least squares; mITT=modified intent-to-treat; max=maximum; min=minimum; MMRM=mixed-effect model repeated measures; SD=standard deviation; SEM=standard error of the mean.

Notes: mBMI = BMI (kg/m^2) × Albumin (g/L)

In the MMRM model, the outcome variable is change from baseline in mBMI. The model includes baseline mBMI as covariate and fixed effect terms including treatment group, visit, treatment-by-visit interaction, baseline NIS, genotype, age at hATTR symptom onset, previous tetramer stabilizer use and region.

[a] Baseline is defined as the measurement closest to and prior to the first dose of study drug.

[b] LS means, SEM, differences in LS means, 95% CIs, and Day 547 (Month 18) p-value from MMRM model.

Autonomic symptoms questionnaire (Composite Autonomic Symptom Score [COMPASS 31]) change from baseline over time

The COMPASS 31 is a measure of autonomic neuropathy symptoms. The range of possible scores is 0 to 100. A decrease in COMPASS 31 from baseline indicates improvement in autonomic neuropathy symptoms, and an increase from baseline indicates worsening. As seen in the following table, copied from the submission, there was an improvement in mean COMPASS 31 score in the patisiran group at 18 months, compared to mean worsening in the placebo group. *This result is consistent with the positive finding of the primary endpoint.*

Table 16: COMPASS 31 Change from Baseline Over Time, MMRM Model (mITT Population). Source: Study 004 CSR, p. 127).

Visit	Actual/ Change	Statistic[b]	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Baseline[a]	Actual	N	76	146
		Mean	30.31	30.61
		SD	16.366	17.576
		Median	32.13	32.20
		Min, Max	0.0, 69.6	0.0, 72.2
Month 18	Actual	N	54	138
		Mean	33.11	25.61

Visit	Actual/ Change	Statistic[b]	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
		SD	17.58	17.05
		Median	34.88	23.43
		Min, Max	2.2, 70.0	0.0, 64.3
	Change	N	53	136
		Mean	4.55	-4.04
		SEM	2.211	1.279
		Median	3.62	-3.44
		Min, Max	-34.8, 35.0	-45.2, 40.6
		LS Mean (SEM)	2.24 (1.940)	-5.29 (1.300)
		95% CI	-1.59, 6.06	-7.85, -2.72
		LS Mean (SEM) Difference (Patisiran - Placebo)		**-7.53 (2.213)**
		95% CI		**-11.89, -3.16**
		p-value		**0.0008**

Abbreviations: CI-confidence interval; COMPASS 31=composite autonomic symptom score; LS=least squares; max=maximum; min=minimum, mITT=modified intent-to-treat; MMRM=mixed-effect model repeated measures; NIS=neurologic impairment score; SD=standard deviation; SEM=standard error of the mean

In the MMRM model, the outcome variable is change from baseline in COMPASS-31 total score. The model includes baseline score as covariate and fixed effect terms including treatment group, visit, treatment by-visit interaction, baseline NIS, genotype, age at hATTR symptom onset, previous tetramer stabilizer use and region.,

[a] Baseline is defined as the measurement closest to and prior to the first dose of study drug.

[b] LS Means, SEM, Differences in LS Means, 95% CIs and Month 18 p-value from MMRM model.

Exploratory Endpoints

Results of exploratory endpoints of Study 004 are presented descriptively. The applicant's verbatim descriptions of the various exploratory outcome measures are provided below, and are confirmed to be accurate by this reviewer.

The exploratory endpoints were the difference between the patisiran-LNP and placebo groups in the change from baseline in the following measurements at 18 months:

<u>Neurological Impairment Score + 7 (NIS+7)</u>

The NIS+7 is a measure of neurologic impairment. It includes the full NIS, sum of five NCS (different from the Σ5 NCS calculated for mNIS+7), Vibration Detection Threshold (VDT), and heart rate response to deep breathing [HRdb]). The NIS+7 is scored from 0 (no impairment) to 270 points (maximum impairment). As seen in the following table, copied from the submission,

there was a smaller increase in mean NIS+7 score in the patisiran group at 18 months compared to the placebo group. *This result is consistent with the positive finding of the primary endpoint.*

Table 17: NIS+7 Change from Baseline to 18 Months, MMRM Model (mITT Population). Source: Study 004 CSR, p. 131.

Visit[a]	Actual/ Change	Statistic[b]	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Baseline	Actual	N	77	148
		Mean	73.09	77.28
		SD	34.149	36.067
		Median	75.08	75.36
		Min, Max	15.7, 145.6	16.4, 155.6
Month 18	Actual	N	58	138
		Mean	98.89	79.41
		SD	43.42	40.07
		Median	100.00	75.57
		Min, Max	21.1, 174.5	12.7, 179.5
	Change	N	58	138

Visit[a]	Actual/ Change	Statistic[b]	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
		Mean	27.28	3.27
		SEM	2.730	1.357
		Median	23.44	1.68
		Min, Max	-5.1, 85.1	-32.1, 61.4
		LS Mean (SEM)	25.04 (2.250)	0.89 (1.542)
		95% CI	20.60, 29.47	-2.14, 3.93
		LS Mean (SEM) Difference (Patisiran - Placebo)		**-24.14 (2.617)**
		95% CI		**-29.30, -18.98**

Abbreviations: CI=confidence interval; HRdb=heart rate variability with deep breathing; max=maximum; min=minimum; mITT=modified intent-to-treat; MMRM=mixed-effect model repeated measures; NIS + 7=Neurologic Impairment Score +7; NCS=nerve conduction studies; SD=standard deviation; SEM=standard error of the mean; VDT=vibration detection threshold

In the MMRM model, the outcome variable is change from baseline in NIS+7. The model includes baseline score as covariate and fixed effect terms including treatment group, visit, treatment-by-visit interaction, genotype, age at hATTR symptom onset, previous tetramer stabilizer use and region.

Note: The sum of the 7 nerve test scores is calculated as (the mean of NCS sum 5, VDT, and HRdb) multiplied by 7.

[a] Baseline, Month 9, and Month 18 are the averages of two assessments performed at least 24 hours but no more than 7 days apart.

[b] LS Means, SEM, Differences in LS Means, and 95% CIs from MMRM model.

Large and small nerve fiber function:

Large nerve fiber function is defined as the sum of the following components of the mNIS+7 and NIS+7: NCS Σ5 + VDT + QST-BSA_TP. The large fiber function is scored from 0 (no large fiber impairment) to 52 points (maximum large fiber impairment). Small fiber function is defined as the sum of the following components of the mNIS+7 and NIS+7: QST-BSAHP + HRdB + postural BP. The small fiber function is scored from 0 (no small fiber impairment) to 44 points (maximum small fiber impairment).

As seen in the following table, copied from the submission the patisiran-LNP group showed a decrease in the large and small fiber function scores at 18 months, indicating improvement, compared to an increase in the score of the placebo group. *This result is consistent with the positive finding of the primary endpoint.*

Table 18: Small and Large Nerve Fiber Change from Baseline to 18 months, MMRM Model (mITT Population). Source: Study 004 CSR, p. 131.

			Large Nerve Fiber		Small Nerve Fiber	
Visit[a]	**Actual/ Change**	**Statistic[b]**	**Placebo (N=77)**	**Patisiran-LN 0.3 mg/kg (N=148)**	**Placebo (N=77)**	**Patisiran-LNP 0.3 mg/kg (N=148)**
Baseline	Actual	N	77	148	77	148
		Mean	22.11	23.30	13.24	14.71
		SD	10.38	10.98	11.56	12.72
		Median	22.50	22.00	1.32	1.05
		Min, Max	4.0, 51.0	3.0, 52.0	10.00	11.00
Month 18	Actual	N	56	138	56	138
		Mean	26.92	20.56	15.47	9.99
		SD	10.88	10.29	11.95	9.80
		Median	26.00	20.00	14.50	7.00
		Min, Max	7.5, 51.0	1.5, 51.0	0.0, 43.0	0.0, 43.0

			Large Nerve Fiber		Small Nerve Fiber	
Visit[a]	**Actual/ Change**	**Statistic[b]**	**Placebo (N=77)**	**Patisiran-LN 0.3 mg/kg (N=148)**	**Placebo (N=77)**	**Patisiran-LNP 0.3 mg/kg (N=148)**
	Change	N	56	138	56	138
		Mean	4.03	-2.47	2.54	-4.52
		SEM	1.30	0.63	1.16	0.79
		Median	3.75	-1.25	1.00	-2.00
		Min, Max	-29.0, 36.0	-34.0, 14.0	-23.5, 26.5	-34.0, 35.0
		LS Mean (SEM)	4.04 (0.99)	-2.76 (0.67)	3.35 (0.99)	-3.62 (0.68)
		95% CI	2.09, 5.99	-4.08, -1.44	1.39, 5.30	-4.96, -2.29
		LS Mean (SEM) Difference (Patisiran - Placebo)		**-6.80 (1.13)**	-	**-6.97 (1.12)**
		95% CI		**-9.02, -4.59**	-	**-9.19, -4.76**

Abbreviations: CI=confidence interval; LS=least squares; max=maximum; min=minimum; mITT=modified intent-to-treat; MMRM=mixed-effect model repeated measures; SD=standard deviation; SEM=standard error of the mean.
In the MMRM model, the outcome variable is change from baseline in the nerve function parameter.
The model includes baseline score as covariate and fixed effect terms including treatment group, visit, treatment-by-visit interaction, baseline NIS, genotype, age at hATTR symptom onset, previous tetramer stabilizer use and region.
[a] Baseline, Month 9, and Month 18 are the averages of two assessments performed at least 24 hours but no more than 7 days apart.
[b] LS Means, SEM, Differences in LS Means, and 95% CIs from MMRM model.

Grip strength

As seen in the following table, copied from the submission, grip strength in the dominant arm evaluated at 18 months showed a smaller mean decline in the patisiran group than in the placebo group. The declines in grip strength observed in the placebo-group are consistent with the expected natural decline in untreated patients. *This result is consistent with the positive finding of the primary endpoint.*

Table 19: Analysis of Mean Change from Baseline at 18 Months in Grip Strength (kg) Test, MMRM Model (mITT Population). Source: Study 004 CSR, p. 142.

Visit[a]	Actual/ Change	Statistic[b]	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Baseline	Actual	N	77	148
		Mean	17.8	18.4
		SD	10.67	13.57
		Median	15.4	16.4
		Min, Max	0, 47	0, 84
Month 18	Actual	N	56	135
		Mean	10.1	18.1
		SD	9.04	12.65
		Median	8.0	17.7
		Min, Max	0, 35	0, 70
	Change	N	56	135
		Mean	-7.9	-1.0
		SEM	0.92	0.74
		Median	-6.8	0.1
		Min, Max	-32, 5	-47, 34
		LS Mean (SEM)	-7.6 (0.89)	-0.4 (0.62)
		95% CI	-9.3, -5.8	-1.6, 0.8
		LS Mean (SEM) Difference (Patisiran - Placebo) (kg)	-	**7.2 (1.01)**
		95% CI	-	**5.2, 9.2**

Abbreviations: CI=confidence interval; hATTR=hereditary transthyretin-mediated; LS=least squares; max=maximum; min=minimum; mITT=modified intent-to-treat; MMRM=mixed-effect model repeated measures; NIS=neurologic impairment score; SD=standard deviation; SEM=standard error of the mean.
Note: The unit for grip strength test is kg.
In the MMRM model, the outcome variable is change from baseline in grip strength test result. The model includes baseline result as covariate and fixed effect terms including treatment group, visit, treatment-by-visit interaction, baseline NIS, genotype, age at hATTR symptom onset, previous tetramer stabilizer use and region
[a] Baseline, Month 9, and Month 18 are the averages of two assessments performed at least 24 hours but no more than 7 days apart.
[b] LS Means, SEM, Differences in LS Means, and 95% CIs from MMRM model.

EuroQOL (EQ-5D) questionnaire

The EQ-5D is a patient-reported measure of quality of life based on mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. The score ranges from 0 to 1, with 0 being worst and 1 indicating no impairment. The EQ-VAS is the patient's own impression of overall health on a scale of 0 (worst possible health) to 100 (best possible health).

As seen in the following table, copied from the submission, the patisiran-LNP group showed increases in EQ-5D and EQ-VAS scores at 18 months compared to decreases in the scores of the placebo group. *This result is consistent with the positive finding of the primary endpoint.*

Table 20: EQ-5D and EQ-VAS Change from Baseline at 18 Months, MMRM Model (mITT Population): Source: Study 004 CSR, p. 138.

			EQ-5D		EQ-VAS	
Visit	**Actual/ Change**	**Statistic[b]**	**Placebo (N=77)**	**Patisiran 0.3 mg/kg (N=148)**	**Placebo (N=77)**	**Patisiran 0.3 mg/kg (N=148)**
Baseline[a]	Actual	N	76	148	76	148
		Mean	0.65	0.62	54.6	55.7
		SD	0.17	0.18	17.97	19.98
		Median	0.66	0.65	50.00	57.50
		Min, Max	0.18, 1.0	0.13, 0.88	10, 90	5, 92
Month 18	Actual	N	56	138	56	138
		Mean	0.47	0.64	47.8	57.0
		SD	0.24	0.22	20.73	21.55
		Median	0.47	0.68	50.00	60.00
		Min, Max	0.03, 0.88	-0.11, 1.00	5, 90	0, 100
	Change	N	55	138	55	138
		Mean	-0.20	0.01	-9.4	0.10
		SEM	0.03	0.01	2.59	1.51
		Median	-0.18	0.00	-10.0	0.00
		Min, Max	-0.63, 0.15	-0.54, 0.51	-60, 30	-45, 55
		LS Mean (SEM)	-0.17 (0.02)	0.03 (0.02)	-7.1 (2.32)	2.4 (1.55)
		95% CI	-0.21, -0.12	-0.00, 0.059	-11.7, -2.5	-0.60, 5.50
		LS Mean (SEM) Difference (Patisiran - Placebo)	-	**0.20 (0.03)**	-	**9.5 (2.65)**
		95% CI	-	**0.15, 0.25**	-	**4.30, 14.80**

Abbreviation: CI=confidence interval; EQ-5D=European Quality of Life-5 Dimensions; EQ-VAS=EuroQoL visual analogue scale; hATTR=hereditary transthyretin-mediated; max=maximum; min=minimum; mITT=modified intent-to-treat; MMRM=mixed-effect model repeated measures; NIS=neurologic impairment score; SD=standard deviation; SEM=standard error of the mean.
In the MMRM model, the outcome variable is change from baseline in EQ-5D or EQ-VAS score.
The model includes baseline score as covariate and fixed effect terms including treatment group, visit, treatment-by-visit interaction, baseline NIS, genotype, age at hATTR symptom onset, previous tetramer stabilizer use and region.
[a] Baseline is defined as the measurement closest to and prior to the first dose of study drug.
[b] LS Means, SEM, Differences in LS Means, and 95% CIs from MMRM model.

Pathologic evaluation of sensory and autonomic innervation through voluntary skin punch biopsies and analysis of intraepidermal nerve fiber density (IENFD), sweat gland nerve fiber density (SGNFD), and dermal amyloid content.

Dermal amyloid burden is expressed as the percent of area of the skin affected by amyloid. In the distal thigh at 18 months, the patisiran-LNP group LS mean absolute change from baseline was +0.04%, and in the placebo group the LS mean absolute change from baseline was +1.00%. In the distal leg at 18 months, the patisiran-LNP group the LS mean absolute change from baseline was + 0.011%, and in the placebo group the LS mean absolute change from baseline was + 2.152%.

For IENFD at 18 months, there was less worsening? from baseline in the patisiran-LNP group than in the placebo group (Distal thigh: LS mean change from baseline of -2.52 fibers/mm compared with -5.08 fibers/mm, respectively; Distal Leg: LS mean change from baseline: -0.85 and -2.43 fibers/mm, respectively).

For SGNFD at 18 months, there was less worsening? from baseline in the patisiran-LNP group than in the placebo group (Distal thigh: the LS mean change from baseline was -1.15 and -1.21 m/mm3 in the patisiran-LNP and placebo groups, respectively: Distal leg: the LS mean change from baseline was -0.95 and -1.85 m/mm3 in the patisiran-LNP and placebo groups, respectively).

These results are consistent with the positive finding of the primary endpoint.

Assessment of ambulation through FAP stage and Polyneuropathy Disability (PND) score;

The following tables, copied from the submission, show the changes in FAP stage and PND score at 18 months in the patisiran group and in the placebo group. Greater percentages of patients in the patisiran group had improvements in both measures than in the placebo group. *This result is consistent with the positive finding of the primary endpoint.*

Table 21: Summary of FAP Stage Change from Baseline at Month 18 (mITT Population). Source: Study 004 CSR, p. 146.

Visit	Actual/ Comparison	Stage/ Score	Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Baseline [1]		0	0	0
		I	37 (48.1)	67 (45.3)
		II	39 (50.6)	81 (54.7)
		III	1 (1.3)	0
		Missing	0	0
Month 18	Comparison	Worsened	21 (27.3)	21 (14.2)
		No Change	34 (44.2)	112 (75.7)
		Improved	0	5 (3.4)
		Missing	22 (28.6)	10 (6.8)

Abbreviations: FAP=Familial Amyloidotic Polyneuropathy; mITT=modified intent-to-treat.
[a] Baseline is defined as the measurement closest to and prior to the first dose of study drug.

Table 22: Summary of PND Score Change from Baseline at Month 18 (mITT Population). Source: Study 004 CSR, p. 144.

Visit	Actual/ Comparison	Stage/ Score	Placebo (N=77 N (%)	Patisiran-LNP 0.3 mg/kg (N=148) N (%)
Baseline[a]	Actual score	0	0	0
		I	20 (26.0)	36 (24.3)
		II	23 (29.9)	43 (29.1)
		IIIA	22 (28.6)	41 (27.7)
		IIIB	11 (14.3)	28 (18.9)
		IV	1 (1.3)	0
		Missing	0	0
Month 18	Comparison	Worsened	32 (41.6)	30 (20.3)
		No Change	23 (29.9)	96 (64.9)
		Improved	0	12 (8.1)
		Missing	22 (28.6)	10 (6.8)

Abbreviations: LS=least squares; mITT=modified intent-to-treat; PND= Polyneuropathy Disability
[a] Baseline is defined as the measurement closest to and prior to the first dose of study drug.

Cardiac assessment through echocardiogram, troponin I, and N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) levels

The following tables, copied from the submission, show the changes in the above cardiac assessments at 18 months. Note that troponin I could not be accurately assessed because about 90% of troponin I values were reported as <0.1 μg/L based on assay sensitivity, and all such values were imputed to 0.1 μg/L for analysis.

Table 23: Change from Baseline in Echocardiogram Parameters Over Time (Cardiac Subpopulation). Source: Study 004 CSR, p. 153.

			Mean LV Wall Thickness (cm)		LV Mass (g)		Longitudinal Strain (%)		Ejection Fraction (EF) (%)	
Visit	Actual/ Change	Statistic[b]	Placebo (N=36)	Patisiran-LNP 0.3 mg/kg (N=90)	Placebo (N=36)	Patisiran-LNP 0.3 mg/kg (N=90)	Placebo (N=36)	Patisiran-LNP 0.3 mg/kg (N=90)	Placebo (N=36)	Patisiran-LNP 0.3 mg/kg (N=90)
Baseline[a]	Actual	N	36	90	35	90	36	86	36	88
		Mean	1.639	1.682	264.52	275.48	-15.66	-15.13	62.21	60.00
		SD	0.2142	0.2573	77.709	80.109	3.513	3.410	8.607	9.918
		Median	1.615	1.640	243.67	270.94	-15.45	-15.10	62.99	60.64
		Min, Max	1.32, 2.22	1.31, 2.59	153.9, 433.8	155.8, 633.1	-23.6, -9.8	-23.4, -7.4	42.3, 75.7	33.4, 79.7
Month 18	Actual	N	25	79	25	78	25	79	24	79
		Mean	1.620	1.537	266.01	251.26	-14.12	-15.37	61.88	61.99
		SD	0.256	0.270	94.564	79.329	2.859	3.385	8.012	9.295
		Median	1.530	1.520	243.20	250.45	-13.80	-15.80	63.02	63.64
		Min, Max	1.32, 2.28	0.90, 2.27	132.8, 565.2	93.8, 582.4	-18.5, -8.4	-21.9, -8.3	46.3, 74.8	41.0, 76.0
	Change	N	25	79	24	78	25	75	24	77
		Mean	-0.018	-0.106	1.58	-16.14	1.41	0.04	0.46	1.04
		SEM	0.0328	0.0206	10.062	5.602	0.542	0.302	1.408	0.847
		Median	-0.020	-0.100	-0.51	-13.26	1.60	0.30	-0.21	0.20
		Min, Max	-0.39, 0.37	-0.69, 0.38	-80.8, 169.7	-205.4, 162.7	-5.2, 7.5	-6.8, 5.8	-11.8, 16.2	-18.1, 19.6
		LS Mean (SEM)	-0.007 (0.0332)	-0.100 (0.0195)	0.63 (9.427)	-15.12 (5.396)	1.46 (0.481)	0.08 (0.280)	0.57 (1.371)	1.00 (0.768)

			Mean LV Wall Thickness (cm)		LV Mass (g)		Longitudinal Strain (%)		Ejection Fraction (EF) (%)	
Visit	Actual/ Change	Statistic[b]	Placebo (N=36)	Patisiran-LNP 0.3 mg/kg (N=90)	Placebo (N=36)	Patisiran-LNP 0.3 mg/kg (N=90)	Placebo (N=36)	Patisiran-LNP 0.3 mg/kg (N=90)	Placebo (N=36)	Patisiran-LNP 0.3 mg/kg (N=90)
		95% CI	-0.073, 0.059	-0.138, -0.061	-18.05, 19.31	-25.81, -4.42	0.50, 2.41	-0.47, 0.64	-2.15, 3.29	-0.53, 2.52
		LS Mean (SEM) Difference (Patisiran - Placebo)		-0.093 (0.0385)		-15.75 (10.862)		-1.37 (0.557)		0.43 (1.572)
		95% CI		-0.169 -0.017		-37.27, 5.78		-2.48, -0.27		-2.69, 3.55

Abbreviations: CI=confidence interval; LS=least squares; LV=left ventricular; max=maximum; min=minimum; mITT=modified intent-to-treat. MMRM=mixed effect model repeated measures; SD=standard deviation; SEM=standard error of the mean.

In the MMRM model, the outcome variable is change from baseline. The model includes baseline value as covariate and fixed effect terms including treatment group, visit and treatment-by-visit interaction.

[a] Baseline is defined as the measurement closest to and prior to the first dose of study drug.

[b] LS Means, SEM, Differences in LS Means, and 95% CIs from MMRM model.

Table 24: Analysis of Mean Change from Baseline to Month 18 in NT-proBNP (ng/L), MMRM Model. Source: Study 004 CSR, p. 160.

			Cardiac Subpopulation		mITT Population	
Visit[a]	Actual/ Change	Statistic	Placebo (N=36)	Patisiran-LNP 0.3 mg/kg (N=90)	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Baseline	Actual	N	34	88	75	144
		Mean	1318.49	1512.35	1294.37	1246.68
		SD	1468.614	1754.036	2236.144	1787.982
		Median	845.74	756.44	562.81	474.48
		Min, Max	39.9, 6036.4	51.9, 7878.6	24.9, 16497.7	27.9, 9882.4
		Geometric Mean[b]	711.10	726.92	531.29	531.04
		SEM Geometric Mean[b]	151.079	103.015	86.661	59.618
		CV (%) Geometric Mean[b]	190.8	220.3	252.1	226.7
Month 18	Actual	N	24	80	53	137
		Mean	2942.76	1321.74	2184.03	1180.79
		SD	5748.01	1973.96	4136.79	2356.94
		Median	1208.46	626.71	918.09	365.26
		Min, Max	85.8, 28228.4	53.9, 12069.9	54.9, 28228.4	21.0, 20155.1
		Geometric Mean[b]	1116.75	544.09	844.40	417.10
		SEM Geometric Mean[b]	320.757	85.208	166.972	50.645
		CV (%) Geometric Mean[b]	249.8	247.3	263.5	255.7
Month 18	Change	N	23	78	52	134
		Mean	1888.68	55.85	1310.63	103.56
		SEM	985.039	149.442	460.159	118.889
		Median	320.35	-49.90	278.45	-32.94

			Cardiac Subpopulation		mITT Population	
Visit[a]	**Actual/ Change**	**Statistic**	**Placebo (N=36)**	**Patisiran-LNP 0.3 mg/kg (N=90)**	**Placebo (N=77)**	**Patisiran-LNP 0.3 mg/kg (N=148)**
		Min, Max	-467.0, 22191.9	-2286.3, 6871.7	-467.0, 22191.9	-2287.3, 10272.7
	Fold-Change to Baseline	Adjusted Geometric Mean Fold-Change[c]	1.97	0.89	1.91	0.90
		95% CI[c]	1.55, 2.50	0.78, 1.01	1.63, 2.23	0.81, 0.99
		Ratio of Adjusted Geometric Mean Fold-Change (Patisiran/Placebo)[c]		0.45		0.47
		95% CI[c]		0.34, 0.59		0.39, 0.56
		p-value[c]		7.736E-08		7.314E-14

Abbreviations: CI=confidence interval; LS=least squares; max=maximum; min=minimum; MITT=MMRM=mixed effect model repeated measures; NT-proBNP=B-type natriuretic peptide; SD=standard deviation; SEM=standard error of the mean
In the MMRM model, the outcome variable is change from baseline of log-transformed NT-proBNP. The model includes log-transformed baseline value as covariate and fixed effect terms including treatment group, visit, treatment-by-visit interaction.
[a] Baseline is defined as the measurement closest to and prior to the first dose of study drug.
[b] Geometric Means are obtained by exponentially back-transforming the arithmetic mean of log-transformed NT-proBNP. SEM of Geometric Mean is calculated as sqrt (geometric mean^2 * variance on mean of log-transformed data). CV (%) of Geometric Mean is calculated as sqrt(exp(variance of log-transformed data)-1)*100%.
[c] Adjusted Geometric Mean Fold-Change, Ratio of Adjusted Geometric Mean Fold-Change, 95% CIs and Month 18 p-value from MMRM model by exponentially back-transforming LS Means, Difference in LS Means and the corresponding 95% CI.

Reviewer Comment:
The Division of Cardiovascular and Renal Products (DCRP) was consulted regarding the applicant's cardiac endpoints. The following comments are from the DCRP review.

"Study ALN-TTR02-004 does not provide any cardiac efficacy data. Imaging and serum biomarkers such as global longitudinal strain and NT-proBNP do not measure how a patient feels, functions, or survives, nor are they known to predict how a patient feels, functions, or survives and hence do not measure a clinical benefit.

(b) (4)
[redacted]

If one is willing to accept the data selection process in study ALN-TTR02-004, the LV thickness changes were small, and the trend toward improvement in LV strain occurred after the apparent trend in improvement of LVEF (since strain is purported to be the more sensitive indicator of trends in LV systolic function, it should have changed first). The study ALN-TTR02-004 NT-proBNP data were skewed such that their analysis required modification. While that modification suggested a trend toward improvement, there were two open-label extension studies in which echocardiography and NT-proBNP were measured that showed no meaningful differences in these measures with 18 to 24 months of follow-up."

Pharmacodynamic (PD) biomarkers [TTR, retinol binding protein (RBP), vitamin A]

The following table, copied from the submission, shows the serum TTR change from baseline at 18 months.

Table 25: Summary of Serum TTR (ELISA] Percent Reduction (mITT Population). Source: Study 004 CSR, p. 170.

Parameter	Statistic	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
TTR % Reduction at 18 months	N	47	130
	Mean	4.8	84.3
	SEM	3.38	1.48
	Median	6.8	88.7
	Min, Max	-62, 56	-98, 60

For retinol binding protein, the mean serum percent reduction levels were 45% in the patisiran-LNP group and 0.4 % in the placebo group.

For Vitamin A, the mean percent reduction in vitamin A levels was 62% in the patisiran-LNP group and 0.1% in the placebo group.

Reviewer Comment: The observed reduction in TTR in the patisiran group is consistent with the reported mechanism of action for patisiran. See Section 8.5.4 for discussion of any ocular changes that might be associated with reduced Vitamin A levels in patients taking patisiran.

The proportion of patients in the patisiran-LNP and placebo groups who met the pre-defined criterion for rapid disease progression (defined as ≥24-point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline) at 9 months

A greater proportion of patients in the placebo group had rapid disease progression compared with the patisiran-LNP group (6 [7.8%] vs. 1 [0.7%], respectively). *This result is consistent with the positive finding of the primary endpoint.*

Serially evaluate lower limb nerve injury via voluntary magnetic resonance (MR) neurography approximately every 6 months in patients receiving either patisiran-LNP or placebo from France and Germany

The applicant reports that no conclusions can be made from the MR neurography evaluation because data from only 2 placebo patients and 10 patisiran patients were obtained, with

variability in the timing and the number of scans obtained.

Dose/Dose Response

See section 7.1.4.

Patisiran-LNP was administered at a dose of 0.3 mg/kg every three weeks as an IV infusion in Study 004.

Durability of Response

See Section 7.1.5.

Persistence of Effect

See Section 7.1.5.

6.2. Uncontrolled Open-Label Studies

The applicant provided supportive efficacy data from two uncontrolled open-label studies (Study 003 completed; Study 006 ongoing with interim analysis). The studies and their efficacy results are described briefly below. Pooled safety results for the open-label studies are discussed in Section 8.

Reviewer Comment: These open-label studies are difficult to interpret because of the intrinsic limitations of the study design, such as the lack of placebo control groups and the potential for observer bias in some endpoints. The results are reported descriptively. The results may lend supportive efficacy evidence if there is a consistent positive trend across studies and endpoints.

6.2.1. ALN-TTR02-003: A Phase 2, Multicenter, Open-Label, Extension Study to Evaluate the Long-Term Safety, Clinical Activity, and Pharmacokinetics of ALN-TTR02 [patisiran] in Patients With Familial Amyloidotic Polyneuropathy Who Have Previously Received ALN-TTR02

Study 003 Design

This open-label extension study of 27 hATTR patients was conducted at 9 study centers in Europe, the United States (US), and South America. Patients who received and tolerated patisiran in the multiple ascending dose Study ALN-TTR02-002 were eligible to participate. Patients participated in this extension study for up to 2 years and 4 months, which included a 28-day screening period, a 2-year treatment period, and up to a 56-day follow-up period after

the last dose. All patients received 0.3 mg/kg patisiran IV once every 3 weeks. Patients were also administered premedications to reduce the risk of infusion related reaction (IRR), as described in Section 7.1.4. The study included a cardiac subgroup, which underwent serial echocardiograms and blood tests (troponin I, N-terminal pro b-type natriuretic peptide [NT-pro-BNP]).

The main objectives of the study are described by the applicant as follows.

The primary objective was to evaluate the safety of long-term dosing with patisiran.

The secondary objectives included:

- Assessing the pharmacodynamic (PD) effect of long-term dosing of patisiran on serum TTR.
- Assessing changes from baseline in:
  - Neurologic impairment using the modified Neuropathy Impairment Score (mNIS) +7 composite score
  - Quality of life (EQ5D) and disability (Rasch-built Overall Disability Scale [R-ODS])
  - Motor function impacting activities of daily living, including a 10-meter walk test (10-MWT) and test of grip strength
  - Nutritional status (modified body mass index [mBMI])

Study 003 Results

The following figures and tables, copied from the submission, summarize the key efficacy results of Study 003. Transthyretin (TTR) levels were reduced as seen below. *This result is consistent with the reported mechanism of action of patisiran.*

Figure 7: Absolute TTR Mean (± SEM) Levels over Time (Full Analysis Set). Source: Study 003 CSR, p. 75.

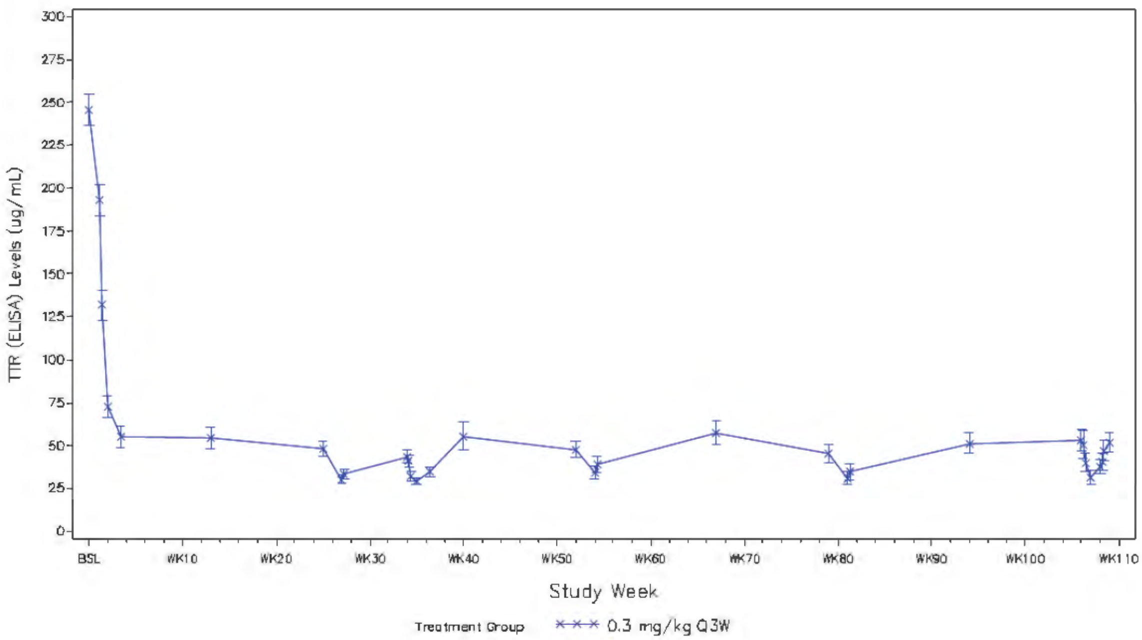


Table 26: Summary of Serum Transthyretin (TTR) Reduction Over 24 Months (Full Analysis Set). Source: Study 003 CSR, p. 76.

	TTR % Reduction (N=27)
Individual Mean TTR Percent Reduction from Baseline over 24 months	
Mean (SEM)	82.06 (1.33)
Min, Max	65.0, 93.3
Individual Mean Predose TTR Percent Reduction from Baseline over 24 months	
Mean (SEM)	79.73 (1.45)
Min, Max	61.4, 93.1
Individual Maximum TTR Percent Reduction from Baseline over 24 months	
Mean (SEM)	92.54 (0.67)
Min, Max	84.6, 96.5

Abbreviations: SEM=standard error of the mean; TTR=transthyretin

The mean Modified Neurologic Impairment Score +7 (mNIS+7) declined over 24 months, as seen in the following table copied from the submission. An increase from baseline in mNIS+7 score suggests worsening of neurological impairment, and a decrease from baseline suggests improvement. *This result is consistent with the results of the placebo-controlled Study 004*. As noted, the interpretability of the efficacy results from an open-label study is challenging. However, the fact that patients demonstrated a mean improvement in mNIS+7 scores is notable, and inconsistent with the natural history of the disease. Although these findings do not provide the primary evidence in support of the effectiveness of patisiran for the treatment of hATTR-PN, they are capable of serving as supportive evidence of the results of Study 004.

Table 27: Summary of mNIS+7 at Baseline, at 24 Months, and Change from Baseline, Full Analysis Set. Source: Study 003 CSR, p. 85.

Visit		Statistic	Overall	Any TTR Stabilizer Use	No TTR Stabilizer Use
Baseline	Actual	N Mean (SD) Median Min, Max	27 53.02 (35.63) 50.5 2.00, 122.50	20 45.31 (30.36) 42.00 2.00, 95.25	7 75.07 (42.61) 52.25 20.50, 122.50
Month 24	Actual	N Mean (SD) Median Min, Max	26 48.04 (33.38) 40.00 3.00, 127.75	19 40.56 (27.02) 39.00 3.00, 99.13	7 68.33 (42.35) 57.00 12.50, 127.75
	Change from Baseline	N Mean (SEM) Median Min, Max	26 -6.95 (2.03) -7.00 -34.63, 15.38	19 -7.03 (2.11) -6.625 -34.63, 3.88	7 -6.75 (5.24) -8.50 -28.50, 15.38

Abbreviations: mNIS=modified Neuropathy Impairment Score; SD=standard deviation; SEM=standard error of the mean; TTR=transthyretin

The results of patient reported quality of life (EQ-5D and EQ-VAS) are shown in the following tables, copied from the submission. *The results are consistent with the results of the placebo-controlled Study 004.* The overall EQ-5D is measured on a scale from 0 to 1, with 0 being worst and 1 best.

Table 28: Summary of EQ-5D at Baseline, at 24 Months, and Change from Baseline, Full Analysis Set. Source: Study 003 CSR, p. 94.

Visit	Actual/ Change	Statistic	Overall	Any TTR Stabilizer Use	No TTR Stabilizer Use
Baseline	Actual	N Mean (SD) Median Min, Max	27 0.78 (0.14) 0.76 0.31, 1.00	20 0.80 (0.04) 0.80 0.31, 1.00	7 0.74 (0.03) 0.73 0.60, 0.85
Month 24	Actual	N Mean (SD) Median Min, Max	26 0.76 (0.16) 0.78 0.28, 1.00	19 0.78 (0.18) 0.80 0.28, 1.00	7 0.72 (0.04) 0.71 0.58, 0.88
	Change	N Mean (SEM) Median Min, Max	26 -0.01 (0.02) 0.00 -0.22, 0.17	19 -0.01 (0.02) 0.00 -0.22, 0.17	7 -0.01 (0.03) 0.00 -0.14, 0.08

Abbreviations: SD=standard deviation; SEM=standard error of the mean; TTR=transthyretin

The EQ-VAS is measured on a scale of 0-100, with 0 being the worst and 100 the best.

Table 29: Summary of EQ-VAS at Baseline, at 24 Months, and Change from Baseline, Full Analysis Set. Source: Study 003 CSR, p. 95.

Visit	Actual/ Change	Statistic	Overall	Any TTR Stabilizer Use	No TTR Stabilizer Use
Baseline	Actual	N Mean (SD) Median Min, Max	27 67.9 (17.85) 70.0 30, 98	20 69.7 (17.41) 72.5 40, 98	7 62.9 (19.55) 70.0 30, 80
Month 24	Actual	N Mean (SD) Median Min, Max	27 69.3 (20.59) 75.0 25, 98	20 69.1 (22.72) 80.0 25, 98	7 70.0 (14.72) 75.0 45, 90
	Change	N Mean (SEM) Median Min, Max	26 1.7 (2.53) 0.0 -25, 30	19 -0.3 (2.31) 0.0 -15, 20	7 7.1 (6.97) 5.0 -25, 30

Abbreviations: EQ-VAS=EuroQoL Visual Analog Scale; SD=standard deviation; SEM=standard error of the mean; TTR=transthyretin

The result of patient reported disability (R-ODS) is shown in the following table, copied from the submission. *This result is consistent with the results of the placebo-controlled Study 004.* R-ODS is measured on a scale of 0-48, with 0 being the worst and 48 the best (no disability).

Table 30: Summary of R-ODS at Baseline, at 24 Months, and Change from Baseline, Full Analysis Set. Source: Study 003 CSR, p. 96.

Visit	Actual/ Change	Statistic	Overall	Any TTR Stabilizer Use	No TTR Stabilizer Use
Baseline	Actual	N Mean (SD) Median Min, Max	26 38.1 (8.61) 38.5 15, 48	19 39.3 (9.57) 42.0 15, 48	7 34.7 (4.03) 34.0 30, 41
Month 24	Actual	N Mean (SD) Median Min, Max	26 36.1 (10.44) 39.0 15, 48	19 37.1 (11.39) 39.0 15, 48	7 33.6 (7.41) 34.0 23, 42
	Change	N Mean (SEM) Median Min, Max	25 -1.8 (0.83) -1.0 -14, 8	18 -2.1 (0.95) 0.0 -14, 2	7 -1.1 (1.79) -2.0 -7, 8

Abbreviations: R-ODS=Rasch-built Overall Disability Scale; SD=standard deviation; SEM=standard error of the mean; TTR=transthyretin

The result of change in modified body mass index (mBMI) over 24 months is shown in the following table, copied from the submission. There is relative stability over the first 12 months, followed by a larger decline through the end of the study. *This result is difficult to interpret without a placebo control. See the results of placebo-controlled Study 004 above.*

Table 31: Change from Baseline in mBMI by 6-month Intervals, Overall, Full Analysis Set. Source: Study 003 CSR, p. 97.

	Patisiran Treatment Overall			
	6 months (Week 25)	12 months (Week 52)	18 months (Week 79)	24 months (Week 109)
N	26	27	25	22
Mean change (SEM)	-0.68 (14.14)	1.94 (21.02)	-32.08 (31.40)	-60.76 (34.86)
Median change (range)	-7.56 (-145.1, 139.4)	8.72 (271.1, 155.1)	9.19 (-318.9, 208.0)	-39.85 (-368.8, 258.9)

Abbreviations: mBMI=modified body mass index; SEM=standard error of the mean

The result of change in 10-meter walk test (gait speed) over 24 months is shown in the following table, copied from the submission. There is relative stability through the end of the study. *This result is consistent with the results of placebo-controlled Study 004 above.*

Table 32: Summary of 10-meter Walk Test (Gait Speed) at Baseline, at 24 Months, and Change from Baseline, Full Analysis Set. Source: Study 003 CSR, p. 98.

Visit	Actual/ Change	Statistic	Overall	Any TTR Stabilizer Use	No TTR Stabilizer Use
Baseline	Actual	N	22	15	7
		Mean (SD)	1.14 (0.79)	1.19 (0.46)	1.05 (0.36)
		Median	1.13	1.18	1.11
		Min, Max	0.4, 2.2	0.4, 2.2	0.7, 1.7
Month 24	Actual	N	26	19	7
		Mean (SD)	1.24 (0.09)	1.32 (0.40)	1.02 (0.47)
		Median	1.30	1.37	1.04
		Min, Max	0.4, 2.1	0.5, 2.1	0.4, 1.8
	Change	N	21	14	7
		Mean (SEM)	0.03 (0.04)	0.06 (0.05)	-0.02 (0.06)
		Median	0.09	0.09	0.04
		Min, Max	-0.4, 0.3	-0.4, 0.3	-0.3, 0.1

Abbreviations: SD=standard deviation; SEM=standard error of the mean; TTR=transthyretin

The result of change in hand grip strength over 24 months is shown in the following table, copied from the submission. There is relative stability through the end of the study. *This result is consistent with the results of placebo-controlled Study 004 above.*

Table 33: Summary of Hand Grip Strength at Baseline, at 24 Months, and Change from Baseline, Full Analysis Set. Source: Study 003 CSR, p. 99.

Visit	Actual/ Change	Statistic	Overall	Any TTR Stabilizer Use	No TTR Stabilizer Use
Baseline	Actual	N Mean (SD) Median Min, Max	27 25.81 (11.86) 23.88 3.2, 49.3	20 27.27 (11.80) 25.82 9.0, 49.3	7 21.65 (11.88) 22.53 3.2, 35.5
Month 24	Actual	N Mean (SD) Median Min, Max	26 27.41 (13.04) 25.08 2.8, 51.5	19 28.67 (12.71) 28.63 7.2, 51.5	7 23.99 (14.34) 19.33 2.8, 44.2
	Change	N Mean (SEM) Median Min, Max	26 1.49 (1.23) 1.54 -17.2, 22.7	19 1.18 (0.53) 1.08 -3.1, 6.0	7 2.34 (4.60) 2.63 -17.2, 22.7

Abbreviations: SD=standard deviation; SEM=standard error of the mean; TTR=transthyretin

The change in COMPASS 31 scores at 24 months is shown in the following table, copied from the submission. *Although this result shows slight worsening compared to the improvement seen in the drug groups of Study 004 and Study 006, this result is difficult to interpret without a concurrent placebo control group.*

Table 34: Summary of COMPASS 31 Score at Baseline, at 24 Months, and Change from Baseline, Full Analysis Set. Source: Study 003 CSR, p. 107.

Visit	Actual/ Change	Statistic	Overall	Any TTR Stabilizer Use	No TTR Stabilizer Use
Baseline	Actual	N Mean (SD) Median Min, Max	27 15.85 (13.34) 9.82 0.0, 46.1	20 14.51 (13.57) 8.95 0.0, 46.1	7 19.68 (12.86) 17.18 3.6, 40.4
Month 24	Actual	N Mean (SD) Median Min, Max	26 16.40 (15.88) 11.67 0.0, 53.1	19 13.93 (15.19) 9.90 0.0, 53.1	7 23.13 (16.93) 14.34 5.8, 49.6
	Change	N Mean (SEM) Median Min, Max	26 1.32 (1.80) 0.11 -15.8, 24.0	19 0.53 (2.04) -1.74 -15.8, 24.0	7 3.45 (3.90) 2.22 14.7, 18.9

Abbreviations: COMPASS 31=Composite Autonomic Symptom Score; SD=standard deviation; SEM=standard error of the mean; TTR=transthyretin

6.2.2. ALN-TTR02-006: A Multicenter, Open-Label, Extension Study to Evaluate the Long-term Safety and Efficacy of Patisiran in Patients with Familial Amyloidotic Polyneuropathy Who Have Completed a Prior Clinical Study with Patisiran

Study 006 Design

This open-label extension study of 188 hATTR patients is being conducted at 44 clinical sites worldwide. Efficacy data are available for 64 patients from the interim analysis at 52 weeks. Patients who completed Studies 003 or 004 are eligible to participate. Patients who enter into this study from Study 004 remain blinded to their treatment allocation (patisiran-LNP or placebo) on Study 004. Efficacy assessments are performed at 52 weeks post-baseline, followed by more limited efficacy assessments yearly thereafter for up to approximately 5 years. All patients receive 0.3 mg/kg patisiran IV once every 3 weeks. Patients are also administered premedications to reduce the risk of infusion related reaction (IRR), as described in Section 7.1.4.

Study 006 includes the following efficacy assessments.

- Neurologic Impairment (mNIS+7, NIS, and NIS+7)
- Quality of Life Measures (Norfolk QOL–DN, EuroQol (EQ-5D-5L and EQ-VAS), Rasch-built Overall Disability Scale (R-ODS))
- Nutritional Status (Modified Body Mass Index, mBMI)
- Timed 10-meter Walk Test (Gait Speed)
- Test of Hand Grip Strength
- Stage of Progression and Ambulation (PND and FAP Stage)
- Pathologic Evaluation of Sensory and Autonomic Innervation and Amyloid Burden (Skin Punch Biopsies)
- Patient Reported Autonomic Neuropathy Symptoms (COMPASS 31)

Study 006 Results

The following figures and tables, copied from the submission, summarize the key efficacy results of Study 006.

<u>Neurologic Impairment (mNIS+7, NIS, and NIS+7)</u>

An increase from baseline in mNIS+7, NIS, and NIS+7 scores suggests worsening of neurological impairment, and a decrease from baseline suggests improvement. *These three scores have overlapping components, and are therefore not independent of each other.* Note that the "004

Placebo" column indicates that this subset of subjects had received placebo during Study 004. *These prior placebo subjects have higher baseline mean scores, indicating greater neurological impairment than subjects who previously received patisiran in Study 004 or 003.* All subjects receive patisiran in Study 006, as described above. As seen in the following tables copied from the submission, *there appears to be relative stability of neurologic impairment, but the result is difficult to interpret without a concurrent placebo control group. The result is consistent with the results of the placebo-controlled Study 004*.

Table 35: <u>Summary of mNIS+7 over Time.</u> The mNIS+7 is scored from 0 (no impairment) to 304 points (maximum impairment). Source: Study 006 CSR, p. 57

mNIS+7 Component	Visit	Actual/ Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
Modified NIS+7	Baseline[a]	Actual	N	43	116	25
			Mean	100.08	77.74	45.66
			SD	43.739	43.695	31.640
	Week 52	Actual	N	10	30	24
			Mean	99.65	81.53	48.49
			SD	44.389	39.167	37.965
			SEM	14.037	7.151	7.749
		Change	N	10	30	24
			Mean	-1.31	1.48	2.47
			SD	9.855	14.019	13.476
			SEM	3.116	2.560	2.751

Abbreviations: mNIS+7=Modified Neuropathy Impairment Score; SD=standard deviation; SEM=standard error of the mean.
Note: Data are from an ongoing study as of 14 July 2017.
Note: Summary statistics are calculated from the mean of the two independent assessments performed at each visit.
[a] The last non-missing measurement on or prior to the first dose of study drug in this study. Per protocol, the last testing visit in the parent study serves as the baseline, unless more than 45 days have elapsed, in which case baseline is the Day 1 value.

Table 36: <u>Summary of NIS Total Score over Time.</u> The NIS is the sum of muscle weakness, reflexes, and sensation in cranial nerves and extremities component scores. The NIS is scored from 0 (no impairment) to 244 points (maximum impairment). Source: Study 006 CSR, p. 58

	Visit	Actual/ Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
Total NIS Score	Baseline[a]	Actual	N	43	116	25
			Mean	81.44	64.67	35.48
			SD	40.962	39.035	28.686
	Week 52	Actual	N	10	30	24
			Mean	81.33	66.55	39.36
			SD	33.163	31.114	31.359
			SEM	10.487	5.681	6.401
		Change	N	10	30	24
			Mean	0.49	1.40	3.37
			SD	10.852	11.116	12.735
			SEM	3.432	2.029	2.599

Abbreviations: NIS=neuropathy impairment score; SD=standard deviation; SEM=standard error of the mean.
Note: Data are from an ongoing study as of 14 July 2017.
[a] The last non-missing measurement on or prior to the first dose of study drug in this study. Per protocol, the last testing visit in the parent study serves as the baseline, unless more than 45 days have elapsed, in which case baseline is the Day 1 value.
Note: Summary statistics are calculated from the mean of the 2 independent assessments performed at each visit.

Table 37: Summary of NIS+7 Composite Score over Time. The NIS+7 includes the full NIS, sum of 5 NCS (different from the Σ5 NCS calculated for mNIS+7), vibration detection threshold (VDT), and heart rate response to deep breathing. The NIS+7 is scored from 0 (no impairment) to 270 points (maximum impairment). Source: Study 006 CSR, p. 59

	Visit	Actual/ Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
NIS+7 Total Score	Baseline[a]	Actual	N	43	116	25
			Mean	98.51	81.28	49.66
			SD	41.500	40.432	31.319
	Week 52	Actual	N	10	30	24
			Mean	98.60	82.51	53.12
			SD	35.389	32.152	34.455
			SEM	11.191	5.870	7.033
		Change	N	10	30	24
			Mean	0.43	1.34	3.30
			SD	11.477	10.356	13.359
			SEM	3.629	1.891	2.727

Abbreviations: NIS=neuropathy impairment score; SD=standard deviation; SEM=standard error of the mean.
Note: Data are from an ongoing study as of 14 July 2017.
Note: Summary statistics are calculated from the mean of the two independent assessments performed at each visit.
[a] The last non-missing measurement on or prior to the first dose of study drug in this study. Per protocol, the last testing visit in the parent study serves as the baseline, unless more than 45 days have elapsed, in which case baseline is the Day 1 value.

Quality of Life Measures (Norfolk QOL–DN, EuroQol (EQ-5D-5L and EQ-VAS), Rasch-built Overall Disability Scale (R-ODS))

The results of patient reported quality of life (Norfolk QOL-DN, EQ-5D, EQ-VAS, R-ODS) are shown in the following tables, copied from the submission. *Note that the prior placebo group from Study 004 has a worse baseline than the groups who previously received patisiran for all of these measures. The results are consistent with the results of the placebo-controlled Study 004.*

The Norfolk QOL-DN is measured on a scale from -4 to 36, with lower scores indicating better quality of life.

Table 38: Summary of Norfolk Quality of Life – Diabetic Neuropathy (Norfolk QOL–DN) Questionnaire over Time. Source: Study 006 CSR, p. 60.

Parameter	Visit	Actual/ Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP[b] (N=25)
Total Score	Baseline[a]	Actual	N	43	116	1
			Mean	73.5	56.0	34.0
			SD	27.69	30.87	NA
	Week 52	Actual	N	10	30	15
			Mean	67.1	53.7	40.7
			SD	31.93	28.54	30.05
			SEM	10.10	5.21	7.76
		Change	N	10	30	0
			Mean	-10.2	-1.8	-
			SD	16.29	10.65	-
			SEM	5.15	1.94	-

Abbreviations: QOL-DN= Quality of Life-Diabetic Neuropathy; SD=standard deviation; SEM=standard error of the mean.
Note: Data are from an ongoing study as of 14 July 2017.
[a] The last non-missing measurement on or prior to the first dose of study drug in this study. Per protocol, the last testing visit in the parent study serves as the baseline, unless more than 45 days have elapsed, in which case baseline is the Day 1 value.
[b] Norfolk QOL was not assessed in the parent study and was not required for these patients in this study.

The overall EQ-5D is measured on a scale from 0 to 1, where 0 is the worst and 1 is the best.

Table 39: Summary of EQ-5D Index Score over Time. Source: Study 006 CSR, p. 61.

Dimension	Visit	Actual/ Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
EQ-5D Index Score	Baseline[a]	Actual	N	43	116	25
			Mean	0.4547	0.6363	0.7663
			SD	0.22516	0.22725	0.16682
	Week 52	Actual	N	10	30	24
			Mean	0.4463	0.6665	0.7468
			SD	0.23781	0.20016	0.16055
			SEM	0.07520	0.03654	0.03277
		Change	N	10	30	24
			Mean	0.0282	0.0094	-0.0179
			SD	0.22575	0.14803	0.10754
			SEM	0.07139	0.02703	0.02195

Abbreviations: EQ-5D=EuroQOL 5 dimensions; SD=standard deviation; SEM=standard error of the mean.
Note: Data are from an ongoing study as of 14 July 2017.
[a] The last non-missing measurement on or prior to the first dose of study drug in this study. Per protocol, the last testing visit in the parent study serves as the baseline, unless more than 45 days have elapsed, in which case baseline is the Day 1 value.

The EQ-VAS is measured on a scale of 0-100, where 0 is the worst and 100 is the best.

Table 40: Summary of EQ–VAS over Time. Source: Study 006 CSR, p. 62.

Dimension	Visit	Actual/ Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
EQ VAS Score	Baseline[a]	Actual	N	43	116	25
			Mean	45.8	57.2	69.1
			SD	20.47	21.30	20.98
	Week 52	Actual	N	10	30	24
			Mean	47.0	49.7	68.9
			SD	27.51	20.30	20.35
			SEM	8.70	3.71	4.15
		Change	N	10	30	24
			Mean	4.5	-1.5	0.6
			SD	24.09	11.40	9.59
			SEM	7.62	2.08	1.96

Abbreviations: EQ-VAS= EuroQoL visual analogue scale; SD=standard deviation; SEM=standard error of the mean.
Note: Data are from an ongoing study as of 14 July 2017.
[a] The last non-missing measurement on or prior to the first dose of study drug in this study. Per protocol, the last testing visit in the parent study serves as the baseline, unless more than 45 days have elapsed, in which case baseline is the Day 1 value.

R-ODS is measured on a scale of 0-48, where 0 is the worst and 48 is the best (no disability).

Table 41: Summary of Rasch–Built Overall Disability Scale (R–ODS) by Visit. Source: Study 006 CSR, p. 63.

Visit	Actual/ Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
Baseline[a]	Actual	N	43	116	25
		Mean	20.4	29.2	36.7
		SD	12.50	12.66	10.29
Week 52	Actual	N	10	30	23
		Mean	22.3	29.2	36.3
		SD	11.09	11.60	11.07
		SEM	3.51	2.12	2.31
	Change	N	10	30	23
		Mean	1.0	-1.3	-1.0
		SD	3.50	4.38	3.55
		SEM	1.11	0.80	0.74

Abbreviations: SD=standard deviation; SEM=standard error of the mean.
Note: Data are from an ongoing study as of 14 July 2017.
[a] The last non-missing measurement on or prior to the first dose of study drug in this study. Per protocol, the last testing visit in the parent study serves as the baseline, unless more than 45 days have elapsed, in which case baseline is the Day 1 value.

Nutritional Status (Modified Body Mass Index, mBMI)

The result of change in modified body mass index (mBMI) over 52 weeks is shown in the following table, copied from the submission. *This result is consistent with the results of placebo-controlled Study 004 above.*

Table 42: Summary of Modified Body Mass Index over Time. Source: Study 006 CSR, p. 64

Visit	Actual/ Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
Baseline[a]	Actual	N	43	116	25
		Mean	876.0	971.5	1002.3
		SD	228.47	225.33	173.80
Week 52	Actual	N	10	28	24
		Mean	880.5	1055.5	1046.2
		SD	218.58	176.39	182.88
		SEM	69.12	33.33	37.33
	Change	N	10	28	24
		Mean	17.0	29.3	42.8
		SD	161.94	77.46	114.34
		SEM	51.21	14.64	23.34

Abbreviations: SD=standard deviation; SEM=standard error of the mean.
Note: Data are from an ongoing study as of 14 July 2017.
Note: mBMI is calculated by multiplying the BMI (kg/m^2) by serum albumin level (g/L).
[a] The last non-missing measurement on or prior to the first dose of study drug in this study. Per protocol, the last testing visit in the parent study serves as the baseline, unless more than 45 days have elapsed, in which case baseline is the Day 1 value.

Timed 10-meter Walk Test (Gait Speed)

The result of change in 10-meter walk test (gait speed) over 52 weeks is shown in the following table, copied from the submission. There is relative stability through the end of the study. *This result is consistent with the results of placebo-controlled Study 004 above.*

Table 43: Summary of 10–Meter Walk Test Speed (m/s) over Time. Source: Study 006 CSR, p. 65.

Visit	Actual/ Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
Baseline[a]	Actual	N	43	116	25
		Mean	0.541	0.848	1.262
		SD	0.3764	0.4977	0.4128
Week 52	Actual	N	11	30	24
		Mean	0.367	0.882	1.212
		SD	0.3216	0.4753	0.4323
		SEM	0.0970	0.0868	0.0882
	Change	N	11	30	24
		Mean	-0.058	-0.055	-0.065
		SD	0.1047	0.1607	0.1917
		SEM	0.0316	0.0293	0.0391

Abbreviations: SD=standard deviation; SEM=standard error of the mean.
Note: Data are from an ongoing study as of 14 July 2017.
Note: Walk speed values are equal to 10 meters divided by the average time taken to complete the two assessments at each visit. The walk speed for patients unable to perform the walk is imputed as 0.
[a] The last non-missing measurement on or prior to the first dose of study drug in this study. Per protocol, the last testing visit in the parent study serves as the baseline, unless more than 45 days have elapsed, in which case baseline is the Day 1 value.

Test of Hand Grip Strength

The result of change in hand grip strength over 52 weeks is shown in the following table, copied from the submission. There is relative stability through the end of the study. *This result is consistent with the results of placebo-controlled Study 004 above.*

Table 44: Summary of Grip Strength Measurements (kg) over Time. Source: Study 006 CSR, p. 66

Visit	Actual/ Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
Baseline[a]	Actual	N	43	116	25
		Mean	10.43	17.74	27.86
		SD	8.548	13.022	13.131
Week 52	Actual	N	10	30	24
		Mean	13.82	16.16	27.63
		SD	8.555	12.172	13.694
		SEM	2.705	2.222	2.795
	Change	N	10	30	24
		Mean	0.91	-1.00	-0.50
		SD	4.027	5.016	3.969
		SEM	1.273	0.916	0.810

Abbreviations: SD=standard deviation; SEM=standard error of the mean.
Note: Data are from an ongoing study as of 14 July 2017.
Note: Summary statistics are calculated from the mean of the two independent assessments performed at each visit.
[a] The last non-missing measurement on or prior to the first dose of study drug in this study. Per protocol, the last testing visit in the parent study serves as the baseline, unless more than 45 days have elapsed, in which case baseline is the Day 1 value.

Stage of Progression and Ambulation (PND and FAP Stage)

The following tables, copied from the submission, show the changes in FAP stage and PND score at 52 weeks. *These results are consistent with the results of placebo-controlled Study 004 above.*

Table 45: Summary of FAP Stage and Comparison with Baseline over Time. Source: Study 006 CSR, p. 69

	Visit	Actual/ Comparison	Statistic	Stage/ Score	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
FAP Stage	Baseline[a]	Actual	N	Total	43	116	25
			N (%)	0	0	0	0
			N (%)	I	12 (27.9)	48 (41.4)	20 (80.0)
			N (%)	II	25 (58.1)	61 (52.6)	5 (20.0)
			N (%)	III	6 (14.0)	7 (6.0)	0
	Week 52	Actual	N	Total	10	30	24
			N (%)	0	0	0	0
			N (%)	I	1 (10.0)	9 (30.0)	18 (75.0)
			N (%)	II	8 (80.0)	19 (63.3)	6 (25.0)
			N (%)	III	1 (10.0)	2 (6.7)	0
		Comparison	N	Total	10	30	24
			N (%)	Worsened	1 (10.0)	3 (10.0)	1 (4.2)
			N (%)	No Change	9 (90.0)	26 (86.7)	23 (95.8)
			N (%)	Improved	0	1 (3.3)	0

Abbreviations: FAP=familial amyloidotic polyneuropathy.
Note: Data are from an ongoing study as of 14 July 2017.
Note: 'Actual' percentages are based on the number of patients with a non-missing value at the indicated visit. 'Comparison' percentages are based on the number of patients with non-missing values at baseline and the indicated visit.
[a] The last non-missing measurement on or prior to the first dose of study drug in this study. Per protocol, the last testing visit in the parent study serves as the baseline, unless more than 45 days have elapsed, in which case baseline is the Day 1 value.

Table 46: Summary of PND Score and Comparison with Baseline over Time. Source: Study 006 CSR, p. 67.

	Visit	Actual/ Comparison	Statistic	Stage/ Score	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
PND Score	Baseline[a]	Actual	N	Total	43	116	25
			N (%)	0	0	0	0
			N (%)	I	5 (11.6)	27 (23.3)	10 (40.0)
			N (%)	II	9 (20.9)	30 (25.9)	13 (52.0)
			N (%)	IIIA	8 (18.6)	28 (24.1)	1 (4.0)
			N (%)	IIIB	15 (34.9)	24 (20.7)	1 (4.0)
			N (%)	IV	6 (14.0)	7 (6.0)	0
	Week 52	Actual	N	Total	10	30	24
			N (%)	0	0	0	0
			N (%)	I	1 (10.0)	5 (16.7)	10 (41.7)
			N (%)	II	0	8 26.7)	9 (37.5)
			N (%)	IIIA	1 (10.0)	7 (23.3)	3 (12.5)
			N (%)	IIIB	7 (70.0)	8 (26.7)	2 (8.3)
			N (%)	IV	1 (10.0)	2 (6.7)	0
		Comparison	N	Total	10	30	24
			N (%)	Worsened	1 (10.0)	5 (16.7)	4 (16.7)
			N (%)	No Change	9 (90.0)	20 (66.7)	19 (79.2)
			N (%)	Improved	0	5 (16.7)	1 (4.2)

Abbreviations: PND=Polyneuropathy Disability.
Note: Data are from an ongoing study as of 14 July 2017.
Note: 'Actual' percentages are based on the number of patients with a non-missing value at the indicated visit. 'Comparison' percentages are based on the number of patients with non-missing values at baseline and the indicated visit.
[a] The last non-missing measurement on or prior to the first dose of study drug in this study. Per protocol, the last testing visit in the parent study serves as the baseline, unless more than 45 days have elapsed, in which case baseline is the Day 1 valne.

Pathologic Evaluation of Sensory and Autonomic Innervation and Amyloid Burden (Skin Punch Biopsies)

As described in the CSR, dermal amyloid burden is expressed as the percent of area of the skin affected by amyloid. A total of 33 patients had at least a single skin punch biopsy at Week 52. Only 2 patients in the Study 004 placebo group had biopsies for Study 006 and were not included in the summary.

At Week 52, mean change from baseline in dermal amyloid burden in the distal thigh

was -5.262% in the 004 patisiran-LNP group and -2.463% in the 003 patisiran-LNP group. At Week 52, mean change from baseline in dermal amyloid burden in the distal leg was -1.262% in the 004 patisiran-LNP group and -4.339% in the 003 patisiran-LNP group.

For intraepidermal nerve fiber density (IENFD) at Week 52, mean change from baseline in the distal thigh was -1.00 fibers/mm in the 004 patisiran-LNP group and -0.53 in the 003 patisiran-LNP group. At Week 52, mean change from baseline in IENFD in the distal leg was -0.45 in the 004 patisiran-LNP group and -1.06 in the 003 patisiran-LNP group.

For sweat gland nerve fiber density (SGNFD) at Week 52, mean change from baseline in the distal thigh was +0.60 m/mm^3 in the 004 patisiran-LNP group and +1.19 in the 003 patisiran-LNP group. At Week 52, mean change from baseline in SGNFD in the distal leg was +0.57 in the 004 patisiran-LNP group and -0.22 in the 003 patisiran-LNP group.

These results are consistent with the results of placebo-controlled Study 004 above.

<u>Patient Reported Autonomic Neuropathy Symptoms (COMPASS 31)</u>

The change in COMPASS 31 scores at 52 weeks is shown in the following table, copied from the submission. *This result is consistent with the results of placebo-controlled Study 004 above.*

Table 47: Summary of Composite Autonomic Symptom Score (COMPASS 31) over Time. Source: Study 006 CSR, p. 71

	Visit	Actual/ Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
Total Score	Baseline[a]	Actual	N	43	116	25
			Mean	35.82	26.13	15.93
			SD	18.038	17.361	15.116
	Week 52	Actual	N	10	30	24
			Mean	33.70	22.42	13.38
			SD	18.088	14.830	11.995
			SEM	5.720	2.708	2.448
		Change	N	10	30	24
			Mean	-10.34	0.00	-1.80
			SD	11.547	10.095	7.644
			SEM	3.652	1.843	1.560

Abbreviations: SD=standard deviation; SEM=standard error of the mean.
Note: Data are from an ongoing study as of 14 July 2017.
[a] The last non-missing measurement on or prior to the first dose of study drug in this study. Per protocol, the last testing visit in the parent study serves as the baseline, unless more than 45 days have elapsed, in which case baseline is the Day 1 value.

7. Integrated Review of Effectiveness

7.1. Assessment of Efficacy Across Trials

The following table compares key efficacy endpoint results between the single placebo-controlled Study 004 and the two open-label studies, 003 and 006. *As can be seen in the table and in the individual study results in Section 6, the results of the open-label studies are generally consistent with the positive results of the placebo-controlled study.*

Table 48: Comparison of Efficacy Results Across Studies. Source: Reviewer summary of submitted results.

Endpoint	Clinical Study		
	Study 004 **Mean Change from Baseline at Month 18**	**Study 003** **Mean Change from Baseline at Month 24**	**Study 006** **Mean Change from Baseline at Month 13 (Week 52, Mean of Groups)**
mNIS+7	Drug: -4.2; Placebo: 27.9 p<0.001	Drug: -6.9	Drug: 0.88
Norfolk QOL-DN	Drug: -6.7; Placebo: 14.4 p<0.001	Not assessed.	Drug: -6
R-ODS	Drug: 0 Placebo: -8.9 p<0.001	Drug: -1.8	Drug: -0.4
10-MWT	Drug: 0.08 Placebo: -0.24 p<0.001	Drug: -0.02	Drug: -0.06
mBMI	Drug: -3.7 Placebo: -119 p<0.001	Drug: -60.8	Drug: 29.7
COMPASS 31	Drug: -5.3 Placebo: 2.2 p=0.0008	Drug: 1.3	Drug: -4.0
Grip strength	Drug: -1.0 Placebo: -7.9	Drug: 1.5	Drug: -0.2
EuroQOL (EQ-5D)	Drug: 0.01 Placebo: -0.2	Drug: -0.01	Drug: 0.01
EQ-VAS	Drug: 0.1 Placebo: -9.4	Drug: 1.7	Drug: 1.2

7.1.1. Primary Endpoints

The change from baseline to Month 18 on the mNIS+7 was the primary endpoint used for the placebo-controlled Study 004. It was also assessed in the open-label Studies 003 and 006. As discussed in Section 6.1.1, the mNIS+7 is an acceptable endpoint, but the results should be considered in the context of the results of the secondary endpoints, particularly the Norfolk QOL-DN.

As seen in the comparison table above, there was worsening of neurological impairment in the placebo group and improvement in the patisiran group, as measured by the change in mNIS+7 score at 18 months ($p<0.001$). Study 003 had a similar improvement in the mNIS+7 score after 24 months, while Study 006 had a slight overall worsening of the score at 13 months that was smaller than the worsening seen in the placebo group of Study 004.

Reviewer Comment: Overall, these results show a clinically meaningful benefit, with a reduction of neurological impairment that is inconsistent with the natural history of hATTR neuropathy.

7.1.2. Secondary and Other Endpoints

The table above shows the comparison of efficacy results across studies, including secondary and other endpoints. Note that only the primary and secondary endpoints for placebo-controlled Study 004, discussed above, were evaluated statistically for this application.

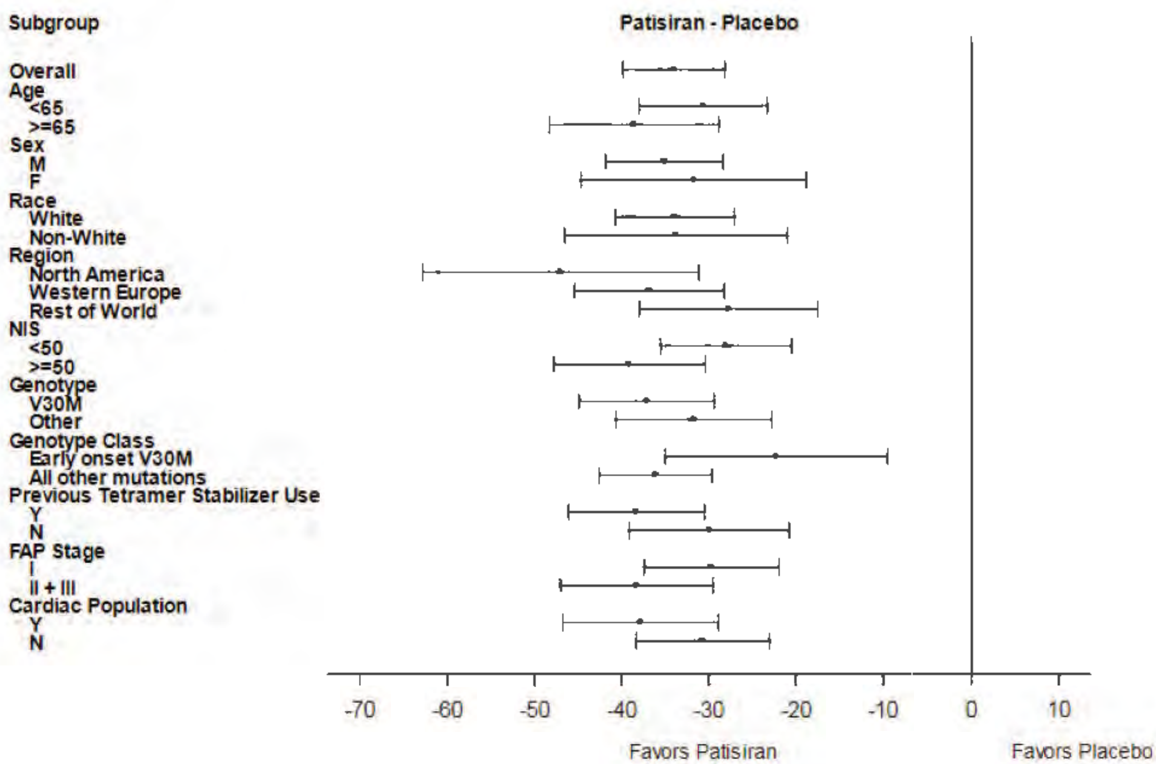
The key secondary endpoint for Study 004 is the change in the Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) score at 18 months. *It is a clinically meaningful endpoint that is appropriate for use in this study.* As discussed in Section 6.1.2 and seen in the table in Section 7.1, there was an improvement in the Norfolk QOL-DN score in the patisiran group, compared to a worsening in the placebo group. There was a similar improvement in the Norfolk QOL-DN score in the patients who received patisiran for 13 months in the open-label Study 006.

All secondary efficacy endpoints for the pivotal placebo-controlled Study 004 had statistically significant results that support the efficacy of patisiran. Overall, the results of the secondary and other endpoints across all studies lend support to the positive efficacy results of the primary endpoint (mNIS+7) for Study 004.

7.1.3. Subpopulations

The efficacy results for the primary endpoint of the placebo-controlled Study 004 are generally similar in the various subpopulations shown in the following figure, copied from the submission.

Figure 8: mNIS+7 Change from Baseline to Month 18 by Subgroup Analysis in Study 004 (mITT Population). Source: Clinical Overview, p. 47



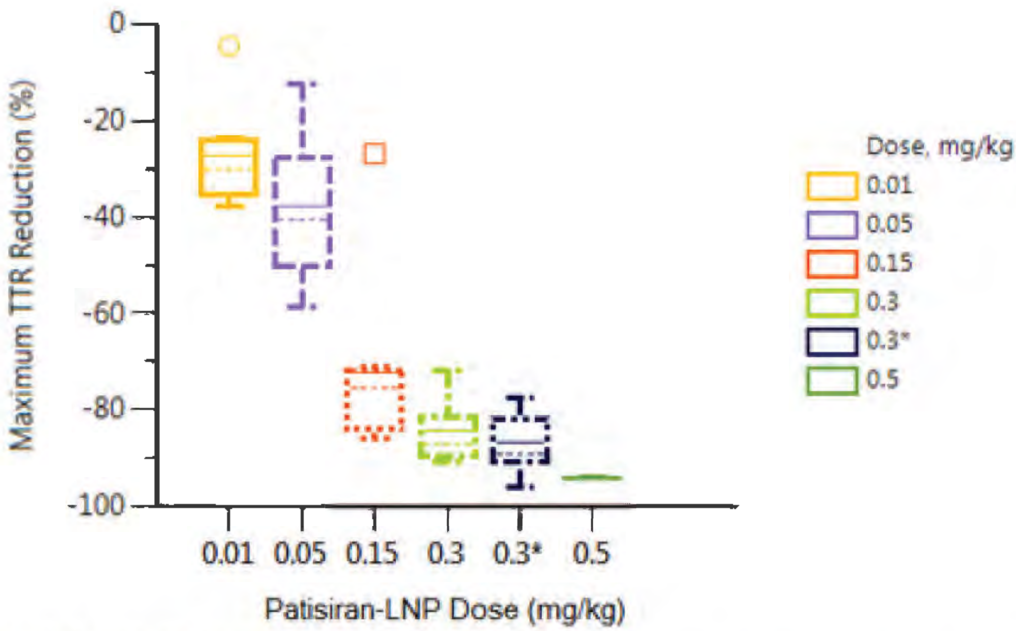
Abreviations: FAP=familial amyloidotic polyneuropathy; LS=least squares; F=female; M=male; mNIS+7= Modified Neurologic Impairment Score +7; V30M=valine to methionine mutation at position 30

7.1.4. Dose and Dose-Response

The applicant's recommended dose for patisiran is 0.3 mg/kg every three weeks as an IV infusion. This recommendation is based on the dose-dependent reduction of TTR levels observed in previous clinical studies, which reflects the mechanism of action of patisiran.

The following figure and table, copied from the submission, show TTR reduction as a function of dose and dosing frequency, respectively, across the development program.

Figure 9: Pooled Analysis for the Relationship between Patisiran-LNP Dose and Maximum Percent TTR Reduction in Dose-Escalation Studies (Studies 001, 002, and 005). Source: Summary of Clinical Efficacy, p. 151.



Abbreviations: TTR=transthyretin
Note: Studies pooled include 001, 002 (first dose) and 005. n=7, 9, 9, 13, 12 and 1 for 0.01, 0.05, 0.15, 0.3 mg/kg dose groups, respectively.
Note: * indicates that patients received reduced premedication.
Note: The ends of the box are the upper and lower quartiles, and the median and mean are marked by the dotted and solid line, respectively, inside the box. The top and lower lines are the range without outliers, and the symbols are outliers.

Table 49: Study 002: Mean (SD) TTR Reduction from Baseline After the First and Second Doses of Patisiran-LNP in Patients. Source: Summary of Clinical Pharmacology, p. 76.

PD Parameter	0.01 mg/kg q4w N=4	0.05 mg/kg q4w N=3	0.15 mg/kg q4w N=3	0.3 mg/kg q4w N=7	0.3 mg/kg q3w N=12
Baseline[a] TTR, µg/mL	272.9 (98.86)	226.5 (12.67)	276.1 (7.65)	242.6 (38.30)	235.5 (44.45)
First Dose					
Maximum percent reduction, %	37.8	58.0	81.7	87.5	94.2
Reduction at Day 21, %	4.6 (12.90)	35.0 (11.94)	55.8 (3.33)	78.2 (8.34)	79.9 (9.22)
Reduction at Day 28, %	8.9 (9.91)	21.7 (1.69)	51.6 (22.84)	73.9 (8.41)	84.9 (8.63)
Second Dose					
Maximum percent reduction, %	34.4	58.5	86.0	90.8	96.0
Reduction at Day 42, %	9.3 (1.77)	24.2 (27.7)	61.2 (10.72)	77.3 (16.88)	74.8 (17.12)
Reduction at Day 56, %	20.3 (20.27)	14.6 (15.80)	44.7 (12.11)	62.8 (25.11)	54.4 (20.04)

Abbreviations: PD=pharmacodynamic(s); q3w=once every 3 weeks; q4w=once every 4 weeks; SD=standard deviation; TTR=transthyretin
Note: Day corresponded to the end of the dosing interval for each regimen (Days 21 [first dose] and 42 [second dose] for q3w dosing interval; Days 28 [first dose] and 56 [second dose] for q4w dosing interval). Percent reduction at Day 28 includes only assessments performed before premedications.
[a] Baseline was defined as the average of all values taken prior to the first study medication dose date and time.

Reviewer Comment: The applicant's recommended dose of patisiran is acceptable based on the above data and the clinical study results. There are do data to evaluate a dose-response with respect to clinical efficacy, as only one dosage level was evaluated in Study 004.

7.1.4.1 Premedication Regimen

In order to reduce the risk of infusion-related reactions during patisiran administration, premedication regimens were used for all clinical studies. The first regimen was used initially, but led to steroid-related SAEs as discussed in Section 8. The regimen was then replaced with the reduced regimen described below.

<u>Original Premedication Regimen</u>
Twelve hours prior to study drug administration, all patients received the following premedication:

- Dexamethasone 8 mg orally (PO) or equivalent;
- Paracetamol 500 mg PO or equivalent;
- H2 blocker PO (e.g., ranitidine 150 mg or famotidine 20 mg or equivalent other H2 blocker dose); and
- H1 blocker PO, 10 mg cetirizine or equivalent (hydroxyzine 25 mg, or fexofenadine, or equivalent could be substituted if patient did not tolerate cetirizine).

On the day of study drug administration, patients also received the following premedication at least 60 minutes prior to the infusion of study drug:

- Intravenous dexamethasone (20 mg) or equivalent;
- Oral paracetamol/acetaminophen (500 mg) or equivalent;
- Intravenous H2 blocker (e.g., ranitidine 150 mg, or famotidine 20 mg, or equivalent other H2 blocker dose); and
- Intravenous H1 blocker; diphenhydramine 50 mg (or equivalent other IV H1 blocker available at study site). Hydroxyzine or fexofenadine 25 mg PO or cetirizine 10 mg PO may be substituted for any patient who does not tolerate IV diphenhydramine or other IV H1 blocker.

Reduced premedication regimen:
There were no premedications administered twelve hours prior to the infusion.

All premedication in the reduced regimen were required at least 60 minutes prior to the start of the infusion of patisiran-LNP:

- Dexamethasone 10 mg IV or equivalent;
- Paracetamol/acetaminophen 500 mg PO or equivalent;
- H2 blocker IV (e.g., ranitidine 50 mg, famotidine 20 mg, or equivalent other H2 blocker dose); and
- H1 blocker IV, diphenhydramine 50 mg (or equivalent other intravenous [IV] H1 blocker available at the study site). Hydroxyzine 25 mg PO or fexofenadine 30 or 25 mg PO or cetirizine 10 mg PO could be substituted for any patient who did not tolerate IV diphenhydramine or other IV H1 blocker.

The original premedication regimen was used in the 2 healthy volunteer studies (ALNTTR02-001 and ALN-TTR02-005). In Study ALN-TTR02-002, patients in the cohorts evaluating dosing every 4 weeks and every 3 weeks with a 60-minute patisiran-LNP infusion received the original regimen and patients in the cohort receiving a slower 80-minute patisiran-LNP infusion every 3 weeks received the reduced premedication regimen. In the three studies ALN-TTR02-003, ALN-TTR02-004, and ALN-TTR02-006, the original regimen was used at the start of studies, but the protocol was subsequently amended to allow patients to switch to the reduced premedication regimen.

7.1.5. Onset, Duration, and Durability of Efficacy Effects

In the placebo-controlled Study 004, stability of mNIS+7 in the patisiran group compared to worsening in the placebo group was seen at 9 months, as shown in the following table, copied from the submission.

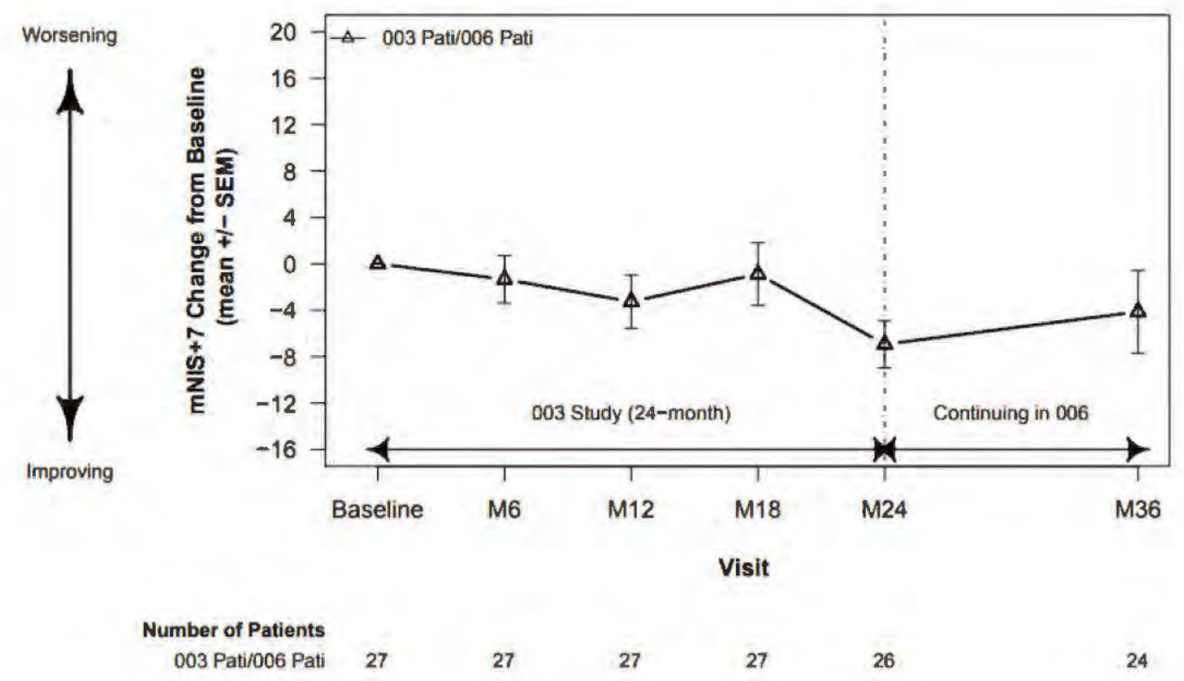
Table 50: mNIS+7 Change from Baseline Over Time, MMRM Model (mITT Population). Source: Study 004 CSR, p. 102.

Visit[a]	Actual/ Change	Statistic[b]	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Baseline	Actual	N	77	148
		Mean	74.61	80.93
		SD	37.041	41.507
		Median	71.50	76.94
		Min, Max	11.0, 153.5	8.0, 165.0
Month 9	Actual	N	67	141
		Mean	90.99	80.12
		SD	41.31	43.26
		Median	91.50	77.50
		Min, Max	19.0, 167.5	10.5, 184.9
	Change	N	67	141
		Mean	15.22	-0.07
		SEM	2.099	1.303
		Median	13.00	0.00
		Min, Max	-16.6, 72.0	-46.3, 46.5
		LS Mean (SEM)	13.95 (2.10)	-2.04 (1.50)
		95% CI	9.80, 18.10	-4.99, 0.91
		LS Mean (SEM) Difference (Patisiran - Placebo)	-	**-15.98 (2.39)**
		95% CI	-	**-20.70, -11.27**
Month 18	Actual	N	51	137
		Mean	101.09	75.13
		SD	45.35	43.18
		Median	93.88	70.63
		Min, Max	21.5, 190.1	8.0, 198.9
	Change	N	51	137
		Mean	27.90	-4.19
		SEM	3.116	1.553
		Median	26.50	-4.00

An improved mNIS+7 score compared to baseline was maintained for up to 36 months in

patients who completed 24 months in Study 003 and then also completed the additional 52 weeks in the long-term extension Study 006, as seen in the following figure copied from the submission.

Figure 10: Change in mNIS+7 Over 36 Months in Patients Treated in Studies 003 and 006. Source: Clinical Overview, p. 51



7.2. Additional Efficacy Considerations

7.2.1. Considerations on Benefit in the Postmarket Setting

There are no additional postmarketing considerations with respect to the established benefit on the treatment of polyneuropathy in patients with hATTR amyloidosis.

7.2.2. Other Relevant Benefits

Based on the mechanism of action of patisiran, it is theoretically possible that it might be beneficial for the non-polyneuropathy symptoms in patients with hATTR. However, any such benefits have not been established in the current development program. Although the applicant included a cardiac subpopulation in the placebo-controlled Study 004 and measured

some cardiac biomarkers, as discussed in Section 6.1, the FDA cardiology consultant concluded that *"Study ALN-TTR02-004 does not provide any cardiac efficacy data"* (b) (4) [redacted]

(b) (4)

[redacted]

(b) (4)

[redacted]

7.3. Integrated Assessment of Effectiveness

The Agency may consider "data from one adequate and well-controlled clinical investigation and confirmatory evidence" to constitute substantial evidence of effectiveness if FDA determines that such data and evidence are sufficient to establish effectiveness (Section 115(a) of the Modernization Act).

The placebo-controlled Study 004 is the one adequate and well-controlled efficacy study that can support approval of patisiran for the treatment of hATTR polyneuropathy. It is a single well-designed multicenter study that has provided reliable and statistically strong ($p=9.3 \times 10^{-24}$) evidence of an important clinical benefit, an effect on polyneuropathy as measured by the Modified Neurological Impairment Score +7 (mNIS+7). Patisiran-treated subjects demonstrated a numerical improvement on mNIS+7 scores from baseline, which is inconsistent with the natural course of the disease. The study has also shown a clinically meaningful and statistically strong ($p<0.001$) improvement in quality of life, as measured by the Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) score as a key secondary endpoint. Further, patisiran showed statistically significant improvement on all other secondary endpoints including motor strength (NIS-W), disability (R-ODS), gait speed (10-MWT), nutritional status (mBMI), and autonomic symptoms (COMPASS 31) at 18 months. The pathophysiology of hATTR amyloidosis and the mechanism of action of patisiran are well understood. Mutant TTR protein causes amyloid deposition, which damages the involved organ systems. Previously, only liver transplant was effective at treating hATTR amyloidosis by reducing mutant TTR production. Pharmacodynamics data for patisiran show a reduction in TTR levels that mirrors the efficacy assessments in a manner consistent with the drug's mechanism of action.

The positive results of the single adequate and well-controlled study are lent further support by descriptive results in two open-label studies (see Section 6.2). The open-label trials, while unable to support approval alone due to their uncontrolled nature, do not contradict the results of Study 004. They provide confirmatory evidence of patisiran's efficacy in the treatment of hATTR polyneuropathy.

8. Review of Safety

8.1. Safety Review Approach

Safety was assessed by evaluating the results from the randomized, double-blind, placebo-controlled Phase 3 trial (ALN-TTR02-004, APOLLO) in patients with hATTR amyloidosis

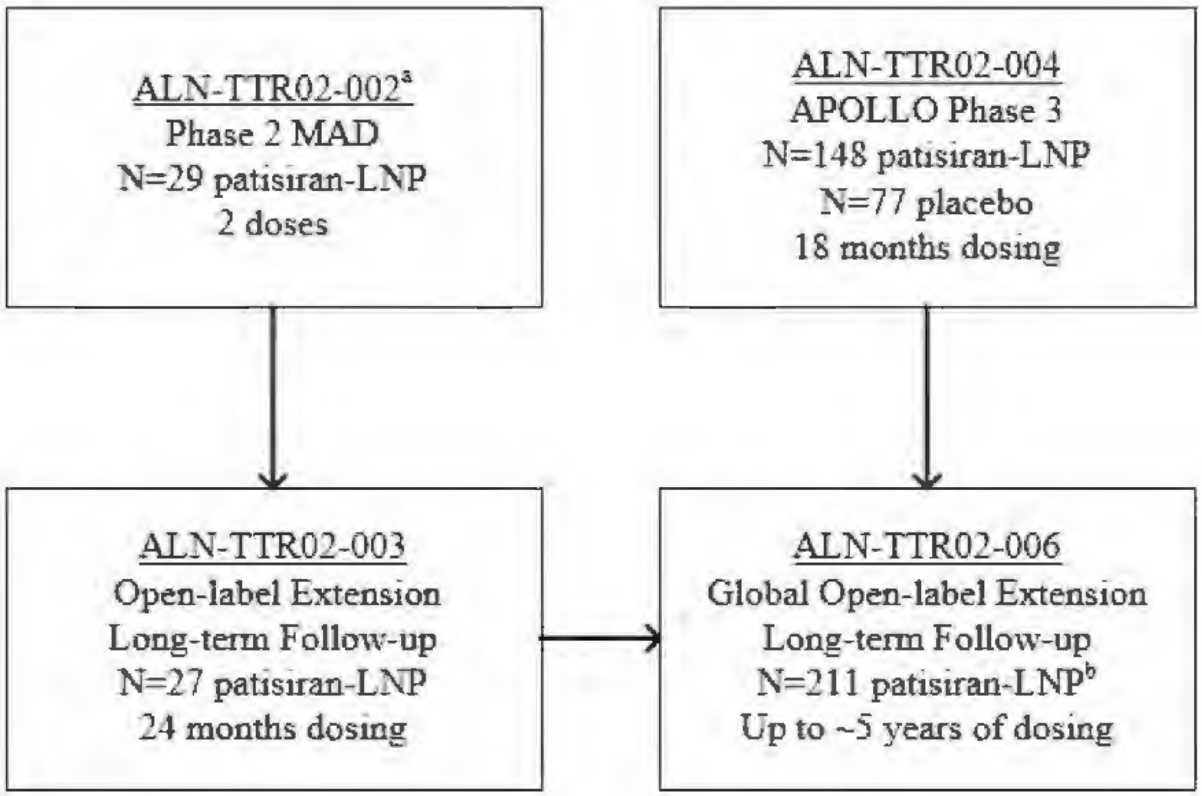
polyneuropathy as well as data from supportive open-label studies (ALN-TTR02-003, -006, and -007). The available narratives for deaths, serious adverse events, laboratory studies, and vital signs were reviewed. Reviewer analyses were conducted on the submitted datasets for the APOLLO trial, including adverse events, serious adverse events, deaths, and laboratory value assessments. The medical scientific literature was searched where appropriate for additional information. The Phase 1 studies (ALN-TTR02-001, -002, and -005) had small populations, short treatment durations, and small drug doses, yielding correspondingly limited safety information and therefore form only a small part of the safety review.

8.2. Review of the Safety Database

8.2.1. Overall Exposure

The size and subject duration of exposure for the patisiran safety population are described in the following tables. A total of 224 patients with hATTR-PN have been exposed to patisiran.

Table 52: Patisiran-LNP Core Clinical Studies and Pooled Safety Experience. Source: Safety Update Report of March 2018



Abbreviations: hATTR=hereditary TTR -mediated amyloidosis; LNP=lipid nanoparticle; MAD=multiple-ascending dose.
[a]Patients were eligible to enter Study 003 if they were dosed in Study 002 (even if they did not complete Study 002).
[b]As of the interim cutoff date of 01 December 2017. Patients remained blinded to their treatment assignment on Study 004 through their 12-month assessments in Study 006.

Table 53: Patisiran Safety Population, Size, and Denominators. Reviewer's assessment

Safety Database for Patisiran Individuals in this development program N= 240 (N is the sum of all available numbers from the columns below)		
Clinical Trial Groups	Patisiran (n= 246)	Placebo (n= 84)
Healthy volunteers (Studies 001 and 005)	22	7
Controlled trials conducted in hATTR amyloidosis patients	148	77

(Study 004)		
All other trials conducted for patisiran in hATTR patients (Studies 002, 003, 006), not including subjects previously in Study 004	76	0

Table 54: Overall Patisiran Exposure, Safety Population. Source: Safety Update Report of March 2018

		Patisiran-LNP 0.3 mg/kg			
Parameter	**Statistic**	**004 Pbo/ 006 Pati (N=49)**	**004 Pati/ 006 Pati (N=148)**	**003 Pati/ 006 Pati (N=27)**	**Total (N=224)**
Total duration of study drug exposure (months)	n	49	148	27	224
	Mean (SD)	10.03 (6.26)	28.01 (8.30)	43.88 (6.65)	25.99 (12.51)
	Median	7.46	28.19	45.89	26.03
	Min, Max	1.3, 24.0	0.7, 47.9	19.3, 49.5	0.7, 49.5
Cumulative study drug exposure (person-years)	Sum	40.9	345.5	98.7	485.2

Table 55: Patisiran Duration of Exposure. Source: Safety Update Report of March 2018

	Number of Patients Exposed to Patisiran:		
Dosage	>=12 months	>=24 months	>= 36 months
Patisiran 0.3 mg/kg	N= 186	N= 137	N= 52

Table 56: Overall Patisiran Exposure, Safety Population. Source: Safety Update Report of March 2018

		Patisiran-LNP 0.3 mg/kg			
Parameter	Statistic	004 Pbo/ 006 Pati (N=49)	004 Pati/ 006 Pati (N=148)	003 Pati/ 006 Pati (N=27)	Total (N=224)
Number of patients on study drug for:					
≥1 day to <3 months	n (%)	3 (6.1)	4 (2.7)	0	7 (3.1)
≥3 months to <6 months	n (%)	15 (30.6)	1 (0.7)	0	16 (7.1)
≥6 months to <9 months	n (%)	9 (18.4)	2 (1.4)	0	11 (4.9)
≥9 months to <12 months	n (%)	4 (8.2)	0	0	4 (8.2)
≥12 months to <15 months	n (%)	8 (16.3)	2 (1.4)	0	10 (4.5)
≥15 months to <18 months	n (%)	3 (6.1)	1 (0.7)	0	4 (1.8)
≥18 months to <21 months	n (%)	3 (6.1)	4 (2.7)	1 (3.7)	8 (3.6)
≥21 months to <24 months	n (%)	3 (6.1)	24 (16.2)	0	27 (12.1)
≥24 months to <27 months	n (%)	1 (2.0)	31 (20.9)	1 (3.7)	33 (14.7)
≥27 months to <30 months	n (%)	0	19 (12.8)	0	19 (8.5)
≥30 months to <33 months	n (%)	0	24 (16.2)	0	24 (10.7)
≥33 months to <36 months	n (%)	0	9 (6.1)	0	9 (4.0)
≥36 months to <39 months	n (%)	0	17 (11.5)	1 (3.7)	18 (8.0)
≥39 months to <42 months	n (%)	0	6 (4.1)	0	6 (2.7)
≥42 months to <45 months	n (%)	0	3 (2.0)	4 (14.8)	7 (3.1)
≥45 months to <48 months	n (%)	0	1 (0.7)	18 (66.7)	19 (8.5)
≥48 months to <51 months	n (%)	0	0	2 (7.4)	2 (0.9)
Total number of doses received	n	49	148	27	224
	Mean (SD)	13.8 (8.8)	39.6 (11.8)	61.0 (9.6)	36.5 (17.7)
	Median	10.0	40.0	64.0	37.0
	Min, Max	2, 34	1, 68	27, 71	1, 71
Cumulative number of doses received	Sum	674	5859	1648	8181

8.2.2. **Relevant Characteristics of the Safety Population:**

The demographic and baseline characteristics for the placebo-controlled and overall pooled populations are summarized in the following tables, copied from the submission. See also Table 9 in Section 6.1.2 for demographic characteristics calculated from the submitted data.

Study 004 enrolled 225 patients. Study 004 enrolled 225 patients. The mean age was 61 years with a median age of 62 years (range 24 to 83 years). 74% of patients were male, 72% were White/Caucasian, and 23% were Asian. Patients were from North America (21%), Western

Europe (44%), and rest of world (ROW) (36%).

Of the thirty-nine different TTR mutations represented, the most common (occurring in 10 or more patients) mutations were Val30Met (42.7%), Ala97Ser (9.3%), Thr60Ala (7.1%), Glu89Gln (6.2%) and Ser50Arg (5.3%). A total of 56% of patients were in the cardiac subpopulation, predefined as patients with baseline LV wall thickness of ≥1.3 cm on echocardiogram indicative of cardiac amyloid involvement, in the absence of other factors that could confound the interpretation of the thickened myocardium, namely, a history of hypertension or aortic valve disease. Fifty-three percent of all patients had a history of prior TTR tetramer stabilizer use.

Table 57: Placebo-controlled Experience: Demographic and Baseline Characteristics (ALN-TTR02-004 Safety Population). Source: Study 004 Summary of Clinical Safety, p. 37

Parameter	Statistic	Placebo (N=77)	Patisiran-LNP (N=148)	Overall (N=225)
Black/African or African American	n (%)	1 (1.3)	4 (2.7)	5 (2.2)
Native Hawaiian or Other Pacific Islander	n (%)	0	0	0
White/Caucasian	n (%)	50 (64.9)	113 (76.4)	163 (72.4)
Other	n (%)	0	1 (0.7)	1 (0.4)
More than one race	n (%)	0	2 (1.4)	2 (0.9)
Missing	n (%)	1 (1.3%)	1 (0.7)	2 (0.9)
Ethnicity				
Hispanic or Latino	n (%)	11 (14.3)	17 (11.5)	28 (12.4)
Not Hispanic or Latino	n (%)	65 (84.4)	130 (87.8)	195 (86.7)
Unknown	n (%)	1 (1.3)	1 (0.7)	2 (0.9)
Region[b]				
North America	n (%)	10 (13.0)	37 (25.0)	47 (20.9)
Western EU	n (%)	36 (46.8)	62 (41.9)	98 (43.6)
Rest of World	n (%)	31 (40.3)	49 (33.1)	80 (35.6)
Eastern EU	n (%)	4 (5.2)	13 (8.8)	17 (7.6)
Asia	n (%)	21 (27.3)	23 (15.5)	44 (19.6)
Central and South America	n (%)	6 (7.8)	13 (8.8)	19 (8.4)
Weight (kg)	Mean (SD)	67.50 (15.72)	67.32 (16.61)	67.38 (16.30)
	Median	67.40	65.00	65.00
	Min, Max	40.8, 99.0	36.2, 110.3	36.2, 110.3
BMI (kg/m2)	Mean (SD)	23.59 (4.27)	22.98 (4.45)	23.18 (4.39)
	Median	23.79	22.76	22.86
	Min, Max	15.1, 35.1	13.9, 36.7	13.9, 36.7
Genotype				
V30M	n (%)	40 (51.9)	56 (37.8)	96 (42.7)
Other	n (%)	37 (48.1)	92 (62.2)	129 (57.3)
Years since diagnosis with hATTR amyloidosis	Mean (SD)	2.60 (3.24)	2.39 (3.26)	2.46 (3.25)
	Median	1.41	1.34	1.37
	Min, Max	0.0, 16.5	0.0, 21.0	0.0, 21.0
Familial Amyloidotic Polyneuropathy (FAP) Stage				
0	n (%)	0	0	0

Parameter	Statistic	Placebo (N=77)	Patisiran-LNP (N=148)	Overall (N=225)
Familial Amyloidotic Polyneuropathy (FAP) Stage				
I	n (%)	37 (48.1)	67 (45.3)	104 (46.2)
II	n (%)	39 (50.6)	81 (54.7)	120 (53.3)
III	n (%)	1 (1.3)	0	1 (0.4)
Polyneuropathy Disability (PND) Score				
I	n (%)	20 (26.0)	36 (24.3)	56 (24.9)
II	n (%)	23 (29.9)	43 (29.1)	66 (29.3)
IIIA	n (%)	22 (28.6)	41 (27.7)	63 (28.0)
IIIB	n (%)	11 (14.3)	28 (18.9)	39 (17.3)
IV	n (%)	1 (1.3)	0	1 (0.4)
New York Heart Association (NYHA) Class				
I	n (%)	40 (51.9)	70 (47.3)	110 (48.9)
II	n (%)	36 (46.8)	77 (52.0)	113 (50.2)
III	n (%)	0	0	0
IV	n (%)	0	0	0
Missing	N (%)	1 (1.3)	1 (0.7)	2 (0.9)
Cardiac Subpopulation[c]	n (%)	36 (46.8)	90 (60.8)	126 (56.0)

Abbreviations: BMI=body mass index; EU=European Union; FAP=familial amyloidotic polyneuropathy; hATTR=hereditary TTR-mediated amyloidosis; LVWT=left ventricular wall thickness; PND=polyneuropathy disability; SD=standard deviation; V30M=Valine to methionine mutation at position 30 in the human transthyretin gene; yrs=years

[a] Patients may be included in more than 1 category.

[b] North America: United States, Canada; Western EU: Germany, Spain, France, Great Britain, Italy, Netherlands, Portugal, and Sweden; Eastern EU: Bulgaria, Cyprus, and Turkey; Asia: Japan, South Korea, and Taiwan; Central and South America: Mexico, Argentina, and Brazil.

[c] cardiac subpopulation: baseline LVWT ≥1.3 cm and no medical history of aortic valve disease or hypertension.

Source: CSR ALN-TTR-004, Tables 14.1.2.1, 14.1.3.1, and 14.1.6.

Table 58: Demographic and Baseline Characteristics (Overall Pooled Experience). Source: Safety Update Report of March 2018

		Patisiran-LNP 0.3 mg/kg			
Parameter	**Statistic**	**004 Pbo/ 006 Pati (N=49)**	**004 Pati/ 006 Pati (N=148)**	**003 Pati/ 006 Pati (N=27)**	**Total (N=224)**
Age at screening (yrs)	Mean (SD)	63.5 (11.0)	59.6 (12.0)	57.9 (15.4)	60.2 (12.3)
	Median	66.0	62.0	64.0	63.0
	Min, Max	36, 78	24, 83	29, 77	24, 83
Age (yrs)					
≥18 to <65	n (%)	22 (44.9)	86 (58.1)	14 (51.9)	122 (54.5)
≥65 to <75	n (%)	20 (40.8)	53 (35.8)	9 (33.3)	82 (36.6)
≥75	n (%)	7 (14.3)	9 (6.1)	4 (14.8)	20 (8.9)
Sex					
Male	n (%)	37 (75.5)	109 (73.6)	18 (66.7)	164 (73.2)
Female	n (%)	12 (24.5)	39 (26.4)	9 (33.3)	60 (26.8)
Race[a]					
Asian	n (%)	14 (28.6)	27 (18.2)	0	41 (18.3)
Black/African or African American	n (%)	0	4 (2.7)	0	4 (1.8)
White/Caucasian	n (%)	34 (69.4)	113 (76.4)	27 (100.0)	174 (77.7)
Other	n (%)	0	1 (0.7)	0	1 (0.4)
More than one race	n (%)	0	2 (1.4)	0	2 (0.9)
Missing	n (%)	1 (2.0)	1 (0.7)	0	2 (0.9)
Ethnicity					
Hispanic or Latino	n (%)	9 (18.4)	17 (11.5)	3 (11.1)	29 (12.9)
Not Hispanic or Latino	n (%)	39 (79.6)	130 (87.8)	24 (88.9)	193 (86.2)
Missing	n (%)	1 (2.0)	1 (0.7)	0	2 (0.9)
Region[b]					
North America	n (%)	5 (10.2)	37 (25.0)	1 (3.7)	43 (19.2)
Western EU	n (%)	26 (53.1)	62 (41.9)	25 (92.6)	113 (50.4)
Rest of World	n (%)	18 (36.7)	49 (33.1)	1 (3.7)	68 (30.4)

		Patisiran-LNP 0.3 mg/kg			
Parameter	Statistic	004 Pbo/ 006 Pati (N=49)	004 Pati/ 006 Pati (N=148)	003 Pati/ 006 Pati (N=27)	Total (N=224)
Eastern EU	n (%)	2 (4.1)	13 (8.8)	0	15 (6.7)
Asia	n (%)	12 (24.5)	23 (15.5)	0	35 (15.6)
Central and South America	n (%)	4 (8.2)	13 (8.8)	1 (3.7)	18 (8.0)
Weight (kg)	Mean (SD)	65.73 (15.19)	67.32 (16.61)	70.75 (15.37)	67.39 (16.15)
	Median	62.80	65.00	72.00	65.50
	Min, Max	42.0, 100.0	36.2, 110.3	42.0, 105.0	36.2, 110.3
BMI (kg/m²)	Mean (SD)	22.87 (4.49)	22.98 (4.45)	24.10 (3.51)	23.09 (4.36)
	Median	22.65	22.76	24.19	22.85
	Min, Max	14.0, 35.4	13.9, 36.7	18.5, 31.4	13.9, 36.7
Genotype					
V30M	n (%)	24 (49.0)	56 (37.8)	20 (74.1)	100 (44.6)
Other	n (%)	25 (51.0)	92 (62.2)	7 (25.9)	124 (55.4)
Years since diagnosis with hATTR amyloidosis	Mean (SD)	4.46 (3.74)	2.39 (3.26)	2.71 (1.40)	2.88 (3.31)
	Median	2.83	1.34	2.56	2.03
	Min, Max	1.7, 18.1	0.0, 21.0	1.1, 8.0	0.0, 21.0
Familial Amyloidotic Polyneuropathy (FAP) Stage					
I	n (%)	14 (28.6)	67 (45.3)	24 (88.9)	105 (46.9)
II	n (%)	27 (55.1)	81 (54.7)	3 (11.1)	111 (49.6)
III	n (%)	8 (16.3)	0	0	8 (3.6)
Polyneuropathy Disability (PND) Score					
I	n (%)	7 (14.3)	36 (24.3)	15 (55.6)	58 (25.9)
II	n (%)	9 (18.4)	43 (29.1)	9 (33.3)	61 (27.2)
IIIA	n (%)	8 (16.3)	41 (27.7)	2 (7.4)	51 (22.8)
IIIB	n (%)	17 (34.7)	28 (18.9)	1 (3.7)	46 (20.5)
IV	n (%)	8 (16.3)	0	0	8 (3.6)

		Patisiran-LNP 0.3 mg/kg			
Parameter	Statistic	004 Pbo/ 006 Pati (N=49)	004 Pati/ 006 Pati (N=148)	003 Pati/ 006 Pati (N=27)	Total (N=224)
New York Heart Association (NYHA) Class					
I	n (%)	22 (44.9)	70 (47.3)	19 (70.4)	111 (49.6)
II	n (%)	21 (42.9)	77 (52.0)	7 (25.9)	105 (46.9)
III	n (%)	4 (8.2)	0	0	4 (1.8)
IV	n (%)	2 (4.1)	0	0	2 (0.9)
Missing	n (%)	0	1 (0.7)	1 (3.7)	2 (0.9)
Cardiac Subpopulation[c]	n (%)	25 (51.0)	90 (60.8)	11 (40.7)	126 (56.3)

Note: The baseline for the pooled analysis was defined as the latest assessment prior to the first dose of patisiran-LNP.
Abbreviations: BMI=body mass index; EU=European Union; FAP=familial amyloidotic polyneuropathy; hATTR=hereditary TTR-mediated amyloidosis; LVWT=left ventricular wall thickness; NYHA=New York Heart Association; pbo=placebo; pati=patisiran-LNP; PND=polyneuropathy disability; SD=standard deviation; V30M=Valine to methionine mutation at position 30 in the human transthyretin gene; yr=years
[a] Patients may be included in more than 1 category.
[b] North America: United States, Canada; Western EU: Germany, Spain, France, Great Britain, Italy, Netherlands, Portugal, and Sweden; Eastern EU: Bulgaria, Cyprus, and Turkey; Asia: Japan, South Korea, and Taiwan; Central and South America: Mexico, Argentina, and Brazil.
[c] 004 Pbo/006 Pati, 004 Pati/006 Pati cardiac subpopulation: baseline LVWT ≥1.3 cm and no medical history of aortic valve disease or hypertension. 003 Pati/006 Pati cardiac subpopulation: baseline LVWT ≥1.3 cm, and, per the Investigator determination, no aortic valve disease or uncontrolled hypertension.

The patient demographics are adequately representative of the range of patients with hATTR-PN that would be exposed to patisiran in clinical practice. Because the cardiac form of hATTR amyloidosis occurs in approximately 25,000 African-Americans, future studies of drugs to treat hATTR-CM should enroll more African-Americans.

8.2.3. Adequacy of the safety database:

For chronically administered drugs, the International Conference on Harmonisation (ICH) E-1 guidelines recommend having studied drug exposure in 1500 patients overall, 300-600 patients for six months, and 100 patients for one year. These exposures must occur at the dose or dose range believed to be efficacious.

When compared to the ICH guidelines, the overall number of exposed subjects is less than the usual recommendation. However, because hATTR amyloidosis is a rare disease, there is no

specific minimum number of patients that should be studied to establish clinical safety. The number of subjects exposed ≥ 1 year exceeds the ICH recommendation.

Because hATTR amyloidosis is a rare and terminal illness, the overall subject exposure in the clinical development program is adequate.

8.3. Adequacy of Applicant's Clinical Safety Assessments

8.3.1. Issues Regarding Data Integrity and Submission Quality

The NDA submission was well-organized. The submission quality with respect to the applicant's clinical safety assessments was acceptable.

8.3.2. Categorization of Adverse Events

The applicant's process for recording AEs was appropriate. The applicant categorized adverse events as mild, moderate, or severe. A subject that experienced the same event more than once was counted only once and at the highest severity for that event. Adverse events were coded to MedDRA 18.0 for Study 004. The FDA analysis reviewed AE coding to assess the accuracy of the translation from the verbatim to the preferred terms. The reviewer grouped related clinical terms together. The following table shows examples of the terms that were grouped in Study 004.

Table 59: Examples of Grouping of Applicant's Preferred Terms for Adverse Events

Verbatim Term	Applicant's Preferred Term	Reviewer's Preferred Term
Synovitis	Synovitis	Tendonitis, Synovitis
Right elbow tendonitis	Tendonitis	Tendonitis, Synovitis
Proteinuria	Proteinuria	Proteinuria, Nephropathy
Parenchymal renal disease, mild, left kidney	Nephropathy	Proteinuria, Nephropathy
Nausea	Nausea	Nausea, Vomiting
Vomiting	Vomiting	Nausea, Vomiting
Accidental fall in the bathroom	Fall	Fall, Dizziness, Balance disorder, Gait disturbance
Imbalance	Balance disorder	Fall, Dizziness, Balance disorder, Gait disturbance

Instability in walking	Gait disturbance	Fall, Dizziness, Balance disorder, Gait disturbance
Bronchitis	Bronchitis	Bronchitis, Bronchiolitis
Bronchiolitis	Bronchiolitis	Bronchitis, Bronchiolitis
Fatigue	Fatigue	Asthenia, Fatigue, Malaise
Asthenia	Asthenia	Asthenia, Fatigue, Malaise

8.3.3. **Routine Clinical Tests**

The safety assessment schedule for the pivotal safety/efficacy Study 004 is summarized in the following tables copied from the study protocol. It consisted of hematology, serum chemistry (including liver function tests), thyroid function parameters, urinalysis, measurement of anti-drug antibodies, electrocardiograms, vital signs, physical examination, and ophthalmology examinations. Assessed pharmacodynamic markers included serum TTR, vitamin A, and retinol binding protein (RBP).

Table 60: Schedule of Assessments: Screening to 9-Month Efficacy Assessment. Source: Study 004 Protocol

Visit Type	Screening (a)	Screening/ Baseline (a)	Baseline (a)	Predosing	Dosing													9-Month Efficacy Assessment
Study Day	Day -42 to 0			D0 Predose	D0	D21	D42	D63	D84	D105	D126	D147	D168	D189	D210	D231	D252	D253-D272
Study Week	NA			0	0	3	6	9	12	15	18	21	24	27	30	33	36	36-39
Procedure / Windows	NA			0	0	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	NA
Informed Consent	X																	
Inclusion/Exclusion Criteria	X	X (a)																
Medical History	X	X (b)	X (b)															
Demographics	X																	
Review Documentation of TTR Genotype	X																	
HIV Status Review	X																	
Karnofsky Performance Status	X																	
New York Heart Classification	X																	
Serology Testing (c)	X																	
Paraprotein by IFE	X																	
Vitamin B12	X																	
Efficacy Assessments																		
NIS (d) / NCS (d)	X																	
mNIS+7 (e)		X	X															X X
NIS+7 (f)		X	X															X X
PND Score and FAP Stage		X (g)	X															X
Skin Punch Biopsy (IENFD and SGNFD) (h)			X															X
mBMI (i)			X															X
10-meter Walk Test (j)			X (k)															X X
Grip Strength Test (k)			X (l)															X X
Norfolk QOL-DN; COMPASS 31 Questionnaires		X (z)																X
EQ-5D; R-ODS Questionnaires			X (l)															X
Echocardiogram			X (l)															X
NT-proBNP and Troponin I			X															X
Pharmacodynamic Assessments (l)																		
TTR Protein, Vitamin A, and RBP			X	X		X					X						X	X
Obtain Blood Sample for Long-term Storage		X	X	X		X					X						X	X
Safety Assessments (m)																		
Physical Examination	X																X	
Weight (n)	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X X
Height	X																	
Vital Signs (o)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X X
12-Lead ECG (p)		X (q)									X							X
Serum Chemistry	X			X					X					X				
Hematology, Urinalysis	X																X	
Thyroid Function Tests	X																X	
Coagulation Studies	X																	
Anti-drug Antibody Testing (r)				X		X					X						X	
Pregnancy Test (s)	X																	
Ophthalmology Exam (t)				X													X	
Concomitant Medications	X	X	X								X							
Adverse Events											X							
Pharmacokinetic Assessments																		
Plasma PK Sampling (u)				X	X	X					X						X	
Urine PK Sampling (u)				X		X					X						X	
Other Assessments																		
Pharmacoeconomic Questionnaire				X (y)														X
C-SSRS Questionnaire		X (z)																X
Drug Administration																		
Randomization (v)				X														
Premedication Administration (w)				X		X	X	X	X	X	X	X	X	X	X	X	X	
Study Drug Administration (x)					X	X	X	X	X	X	X	X	X	X	X	X	X	

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Table 1-1 Footnotes:

Abbreviations: COMPASS 31 = Composite Autonomic Symptom Score; EQ-5D = EuroQOL-5 Dimensions; ECG = electrocardiogram; FAP = familial amyloidotic polyneuropathy; HIV = human immunodeficiency virus; IENFD = Intraepidermal nerve fiber density; IFE = immunofixation electrophoresis; mBMI = modified body mass index; mNIS = Modified Neuropathy Impairment Score; NCS = nerve conduction studies; NIS = Neuropathy Impairment Score; NT-proBNP = N-terminal prohormone of B-type natriuretic peptide; PND = polyneuropathy disability; QOL-DN = Quality of Life-Diabetic Neuropathy; RBP = retinol binding protein; R-ODS = Rasch-built Overall Disability Scale; SGNFD = Sweat gland nerve fiber density; TTR = transthyretin

a. The Screening/Baseline and Baseline visits will be performed on separate days. The Screening/Baseline visit must be performed within 21 days prior to the first dose of study drug (Day 0). The Baseline visit must be conducted at least 24 hours (approximately), but not more than 7 days, after the Screening/Baseline visit. In conjunction with the decision of the Medical Monitor(s), patients may be allowed to rescreen after a minimum of 5 days have elapsed from their last screening assessment. Note: Inclusion Criteria 3 (i.e., NIS of 5 to 130 [inclusive] and PND score ≤3b) and 4 (i.e., NCS sum of the sural sensory nerve action potential [SNAP], tibial compound muscle action potential [CMAP], ulnar SNAP, ulnar CMAP, and peroneal CMAP of ≥2 points) must be met at the Screening/Baseline visit. All other entry criteria (inclusion and exclusion) will be assessed at the Screening visit only.

b. An interval medical history will be collected at the Screening/Baseline and Baseline visit. Only changes since the Screening visit will be collected.

c. Serologies will include hepatitis B surface antibody (HbsAb), hepatitis B surface antigen (HbsAg), and anti-hepatitis C virus antibody (anti-HCV Ab).

d. The NIS and NCS will be assessed for the likelihood of a patient meeting the NIS and NCS eligibility criteria at the Screening/Baseline visit. The documented results of previously performed NIS and NCS may be used to qualify a patient for this study if these tests were performed within 60 days prior to the date of informed consent.

e. The mNIS+7 consists of the modified NIS tool (weakness and reflexes), NCS 5 attributes (Σ5), quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), and postural blood pressure. At the 9-month efficacy assessment, 2 assessments of the mNIS+7 will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart. Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed once at each assessment (e.g., the weakness component should not be performed more than once on any given day).

f. The NIS+7 consists of the NIS tool (weakness, sensation, and reflexes), NCS Σ5, vibration detection threshold (VDT), and heart rate response to deep breathing (HRdB). At the 9-month efficacy assessment, 2 assessments of the NIS+7 will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart. Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed once at each assessment (e.g., the weakness component should not be performed more than once on any given day).

g. At the Screening/Baseline visit, only PND score is required.

h. If the patient has provided separate informed consent for skin biopsies, 2 sets of tandem 3-mm skin punch biopsies are to be obtained (4 biopsies total). One set of biopsies will be taken from the distal lower leg, when a patient's clinical status allows, and one set from the distal thigh at each time point. Skin biopsies will be performed at a central assessment site (CAS).

i. The mBMI calculation will take place programmatically in the clinical database; the site will not perform the calculation.

j. The patient will be asked to walk 10 meters. The walk must be completed without assistance from another person; ambulatory aids such as canes and walkers are permitted. The time required for the patient to walk 10 meters will be recorded. At the 9-month efficacy assessment, 2 assessments of the 10-meter walk test will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart.

k. Grip strength will be measured in triplicate using a dynamometer held in the dominant hand. Every effort will be made to use the same device for a patient throughout the duration of the study. At the 9-month efficacy assessment, 2 assessments of the grip strength will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart.

l. On dosing days, blood samples for PD assessments will be obtained prior to dosing and vitamin A supplementation.

Table 1-1 Footnotes (continued):

m. On dosing days, all safety assessments are performed pre-dose.

n. Weight from previous visit should be used for calculating dose. Weight must be collected pre-dose.

o. Vital signs to include: blood pressure, pulse rate, temperature, and respiratory rate. Parameters are to be measured in the supine position using an automated instrument after the patient has rested comfortably for 10 minutes. Vital signs must be collected pre-dose. On Day 0, vital signs will also be collected post-dose.

p. All ECGs are to be obtained in triplicate.

q. Blood samples for anti-drug antibody testing will be collected prior to study drug dosing.

r. A pregnancy test (urine- or serum-based) will be performed on all females of child-bearing potential.

s. The baseline ophthalmology examination may be performed any time after the patient is deemed eligible for participation in the study through Day 21. The 9-month ophthalmology examination will be performed between Days 231(+3) and 272 at a CAS.

t. Plasma PK samples will be collected as follows: Day 0: pre-dose (within 1 hour of planned study drug dosing) and at the end of infusion (+5 minutes). Day 21 and Day 252: pre-dose (within 1 hour of planned study drug dosing) and 30 minutes after the end of the infusion (+15 minutes). Day 126: pre-dose (within 1 hour of planned study drug dosing) and at the end of infusion (+5 minutes).

u. Urine PK samples will be collected pre-dose (within 1 hour of planned study drug dosing).

v. Randomization procedures are described in Section 4.4.1.

w. Prior to dosing, all patients will receive premedications administered at least 60 minutes prior to the start of infusion of study drug. The regimen is described in Section 5.3.1.

x. The patient's infusion site should be assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion. The patient will remain at the study site for 1 hour following completion of dosing for observation and completion of assessments.

y. Patients who discontinue study drug due to rapid disease progression based on the 9-month efficacy assessments will continue on to the Modified Visit Schedule (See Table 1-3).

z. Assessment must be completed at a single time point during one of the specified visits, at the discretion of the investigator.

Table 61: Schedule of Assessments: Week 39 to Week 86 (Follow-up)/Early Withdrawal. Source: Study 004 Protocol

Procedure	Visit Type	Dosing														18-Month Efficacy Assessment	End of Study[a]	Follow-up[a]	Early Withdrawal[b]	Follow-up for Patients who Discontinue Treatment but Return at 9 and/or 18 mo[c]	
	Study Day	D273	D294	D315	D336	D357	D378	D399	D420	D441	D462	D483	D504	D525	D546	D553-D560	D567	D602	NA	D253-D272	D553-D560
	Study Week	39	42	45	48	51	54	57	60	63	66	69	72	75	78	79-80	81	86	NA	36-39	79-80
	Windows	±3 D	±3 D	±3 D	±3 D	±3 D	±3 D	±3 D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	NA	±7D	±10D	2D to 7D	±10D	±10D
Efficacy Assessments																					
mNIS+7[d]																X X			X X	X X	X X
NIS+7[e]																X X			X X	X X	X X
PND Score and FAP Stage																X			X	X	X
Skin Punch Biopsy (IENFD and SGNFD)[f]																X			X		
mBMI[g]																X			X	X	X
10-meter Walk Test[h]																X X			X X		
Grip Strength Test[i]																X X			X X		
Norfolk QOL-DN; EQ-5D; R-ODS Disability; COMPASS31 Questionnaires																X			X	X[j]	X[j]
Echocardiogram																X			X		
NT-pro BNP and Troponin I																X			X		
Pharmacodynamic Assessments[k]																					
TTR Protein, Vitamin A, and RBP		X						X							X	X	X		X	X	X
Obtain Blood Sample for Long-term Storage		X						X							X	X	X		X		

Procedure	Visit Type: Dosing														18-Month Efficacy Assessment		End of Study[a]	Follow-up[a]	Early Withdrawal[b]		Follow-up for Patients who Discontinue Treatment but Return at 9 and/or 18 mo[c]	
Study Day	D273	D294	D315	D336	D357	D378	D399	D420	D441	D462	D483	D504	D525	D546	D553-D560		D567	D602	NA		D253-D272	D553-D560
Study Week	39	42	45	48	51	54	57	60	63	66	69	72	75	78	79-80		81	86	NA		36-39	79-80
Windows	±3 D	±3 D	±3 D	±3 D	±3 D	±3 D	±3 D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	NA		±7D	±10D	2D to 7D		±10D	±10D
Safety Assessment[f]																						
Physical Examination														X			X	X	X		X	X
Weight[m]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
Vital Signs[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
12-Lead ECG[e]							X								X				X			
Serum Chemistry					X					X				X				X	X			
Coagulation[w]														X								
Hematology, Urinalysis														X					X			
Thyroid Function Tests														X					X			
Anti-drug Antibody Testing[g]							X							X					X			
Pregnancy Test[q]																	X	X	X			
Ophthalmology[r]															X							
Concomitant Medications	X																					
Adverse Events	X																					
Pharmacokinetic Assessments																						
Plasma Pharmacokinetic Sampling[s]							X							X					X		X	X
Urine Pharmacokinetic Sampling[t]							X							X					X			
Other Assessments																						
Pharmacoeconomic Questionnaire															X				X			

Procedure	Visit Type: Dosing														18-Month Efficacy Assessment	End of Study[a]	Follow-up[a]	Early Withdrawal[b]	Follow-up for Patients who Discontinue Treatment but Return at 9 and/or 18 mo[c]	
Study Day	D273	D294	D315	D336	D357	D378	D399	D420	D441	D462	D483	D504	D525	D546	D553-D560	D567	D602	NA	D253-D272	D553-D560
Study Week	39	42	45	48	51	54	57	60	63	66	69	72	75	78	79-80	81	86	NA	36-39	79-80
Windows	±3 D	±3 D	±3 D	±3 D	±3 D	±3 D	±3 D	±3D	±3D	±3D	±3D	±3D	±3D	±3D	NA	±7D	±10D	2D to 7D	±10D	±10D
C-SSRS Questionnaire															X			X		
Drug Administration																				
Premedication Administration[u]	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
Study Drug Administration[v]	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
Karnosky performance status																			X	X
NYHA class																			X	X

Reviewer comment: The clinical testing schedule was adequate. In the opinion of this reviewer, the hematology testing frequency should ideally have matched the more frequent serum chemistry testing frequency.

Here is what this might look like (this one was done in Excel):

	Baseline	In-Hosp	Wk 2	Wk 4	Wk 8	Wk 12	Mo 6	Mo 12	Mo 18, 24, 36, 48, 60	yearly thru year 15
Medical Hx, Stool for occult blood X3, HIV, Hepatitis B, C, Mammogram*, PAP***, Sigmoidoscopy******	X									
Physical exam;* vital signs, Clin Path (blood/urine)**	X		X	X	X	X	X	X	X	
AEs	X	X	X	X	X	X	X	X	X	
EKG; CCS angina per PI	X		X	X	X	X	X	X		
Eye exam -ophthalmologist	X						X			
CXR***	X							X		
PSA (males)	X						X			
FGF-4 serum levels	X	X	X	X						
Ad5.1 neutralizing Ab	X		X	X	X	X	X	X		
Concomitant Meds	X	X	X	X	X	X	X	X		
long-term (postcard) F/U										X##

* includes rectal exam and pelvic exam in females at baseline only; careful skin exam, with derm opinion for undiagnosed lesions
** Includes CBC, diff, platelets, ESR, 'lytes, BUN, Cr, urate, LFTs, protein, albumin, glucose, cholesterol, CPK-MB, troponin T, U/A (dipstick, pH, SG, microscopic)
*** if not done within 12 months
**** if not done within 3 years of study enrollment with negative findings reported by examining MD
\# selected sites: 1, 8 and 24 hours - blood urine, stool and throat.
\#\# **postcard contact:** death, cancer, MI, unstable angina, stroke, neuro disorder,angioplasty, CABG, hematological/autoimmune disorders, other significant illnesses

8.4. Safety Results

The following table summarizes the high-level safety results of the placebo-controlled Study 004, which were confirmed from the submitted data. Additional details are presented in the following sections. *Note that the percentages of deaths, serious, and severe AEs were higher in the placebo group.*

Table 62: Placebo-controlled Experience: Overview of Adverse Events (ALN-TTR02-004 Safety Population). Source: Summary of Clinical Safety, p. 45.

Category	Patisiran 0.3mg/kg n (%) out of N= 148	Placebo n (%) out of N=77
At Least 1 Adverse Event (AE)	143 (96.6)	75 (97.4)
At Least 1 Severe AE	42 (28.4)	28 (36.4)
At Least 1 Serious Adverse Event (SAE)	54 (36.5)	31 (40.3)

At Least 1 AE Leading to Treatment Discontinuation	7 (4.7)	11 (14.3)
At Least 1 AE Leading to Study Withdrawal	7 (4.7)	9 (11.7)
Death	7 (4.7)	6 (7.8)

8.4.1. **Deaths**

There has been a total of 21 deaths in the Patisiran-LNP clinical program, 15 in patisiran-LNP treated patients and 6 in placebo-treated patients, including integrated safety data from the completed Studies 003 and 004 and data as of 7/14/2017 (interim cutoff date) for Study 006.

There was a total of 13 deaths in placebo-controlled study 004, 6 deaths (7.8%) among the 77 patients who received placebo and 7 (4.7%) deaths among the 148 who received patisiran-LNP.

The following table lists the SAEs reported as fatal for the 21 deaths in the patisiran clinical development program.

Table 63: SAEs Reported as Fatal in Patisiran Development. Source: Summary of Clinical Safety, p. 56

Patient Number	Study	Group	Serious Adverse Event reported as Fatal	Independent Adjudication of Cause of Death: Cardiovascular (CV) or Non-CV
(b) (6) [redacted]	004	Patisiran	Cardiac arrest, Cardiac failure congestive	CV (heart failure)
[redacted]	004	Patisiran	Sudden cardiac death	CV (presumed sudden death)
[redacted]	004	Patisiran	Sudden cardiac death	CV (sudden death)
[redacted]	004	Patisiran	Cardiac failure, acute pulmonary edema	CV (sudden death)
[redacted]	004	Patisiran	Cardiac arrest	CV (presumed CV)

(b) (6)

[redacted]	004	Patisiran	Pulseless electrical activity	CV (sudden death)
	004	Patisiran	Cardiac failure	CV (heart failure)
	004	Placebo	Subarachnoid hemorrhage	CV (fatal stroke, hemorrhagic)
	004	Placebo	Staphylococcal sepsis	Non-CV
	004	Placebo	Anemia, Gastrointestinal hemorrhage	CV (heart failure)
	004	Placebo	Acute kidney failure, urinary tract infection, bacteremia	Unknown
	004	Placebo	Colorectal cancer metastatic	Non-CV
	004	Placebo	Ischemic stroke	CV (fatal stroke, ischemic)
	003	Patisiran	Esophageal carcinoma	Non-CV
	003	Patisiran	Myocardial infarction	CV (fatal MI)
	006	004 Placebo/006 Patisiran-LNP Group	Cardiac Arrest	CV (sudden death)
	006	004 Placebo/006 Patisiran-LNP Group	Cardiac Arrest	CV (presumed CV death)
	006	004 Placebo/006 Patisiran-LNP Group	Acute Respiratory Distress Syndrome Hemorrhagic Shock	Non- CV
	006	004 Placebo/006 Patisiran-LNP Group	Cardiogenic Shock	CV (presumed CV death)
	006	004 Patisiran-LNP/006 Patisiran-LNP Group	Invasive ductal breast carcinoma	Non-CV
	006	004 Patisiran-LNP/006 Patisiran-LNP Group	Worsening Amyloidosis	CV (heart failure)

The table in Appendix 13.3, copied from the submission, lists additional details of all the deaths in the patisiran clinical trials.

Reviewer Comment: Although mortality was numerically lower in the patisiran group (4.7%) than in the placebo group (7.8%), Study 004 was not designed to statistically evaluate an effect on survival and no conclusion regarding a potential long-term survival effect can be made. See Section 8.5.7 for a discussion of cardiac deaths in the patisiran and placebo groups of Study 004.

8.4.2. Serious Adverse Events

In the controlled Study 004, the proportion of patients experiencing serious AEs was 37% in the patisiran group and 40% in the placebo group. The following table lists the serious adverse events that occurred in at least 1% of drug or placebo subjects in the Study 004, and occurred in at least 1 patisiran-treated patient. There was more diarrhea in the patisiran group (5.4% vs. 1.3% in placebo). Cardiac SAEs were similar in both patisiran and placebo groups. There was more acute kidney injury in the placebo group (5.2% vs. 0.7% in patisiran). Note that digestive symptoms due to autonomic neuropathy (diarrhea, constipation), cardiac disease (conduction disorders, infiltrative cardiomyopathy), and renal disease (leading to renal failure) are all features of ATTR amyloidosis. All SAEs by system organ class are listed in Appendix 13.4.

In the overall pooled experience over all clinical studies (see the table in Appendix 13.5), SAEs were reported in 40.4% of patients. System organ classes with a frequency of SAEs of ≥5% were cardiac disorders (17.0%), gastrointestinal disorders (7.3%), infections and infestations (6.4%), and vascular disorders (5.5%). SAEs reported in ≥2% of patients were diarrhea (4.1%), cardiac amyloidosis (2.3%), cardiac failure (2.3%), and cardiac failure congestive (2.3%).

Table 64: Serious Adverse Events in Study 004 that occurred in at least 1% of drug or placebo patients. Source: FDA Analysis

	AEDECOD	(N Rows, Patisiran 0.3...	(Percent, Patisiran 0.3 mg/kg, N=148)	(N Rows, PLACEBO, N=77)	(Percent, PLACEBO, N=77)
1	Diarrhoea	8	5.4%	1	1.3%
2	Cardiac failure	3	2.0%	2	2.6%
3	Cardiac failure congestive	3	2.0%	2	2.6%
4	Orthostatic hypotension	3	2.0%	1	1.3%
5	Pneumonia	3	2.0%	3	3.9%
6	Atrioventricular block complete	3	2.0%	•	•
7	Atrial fibrillation	2	1.4%	1	1.3%
8	Cardiac amyloidosis	2	1.4%	1	1.3%
9	Cardiac arrest	2	1.4%	1	1.3%
10	Deep vein thrombosis	2	1.4%	•	•
11	Acute kidney injury	1	0.7%	4	5.2%
12	Conduction disorder	1	0.7%	1	1.3%
13	Dehydration	1	0.7%	3	3.9%
14	Erysipelas	1	0.7%	1	1.3%
15	Pleural effusion	1	0.7%	1	1.3%
16	Skin ulcer	1	0.7%	1	1.3%
17	Vomiting	1	0.7%	3	3.9%

Four (2.7%) of serious adverse events of atrioventricular (AV) heart block, including 3 cases of complete AV block, occurred in the patisiran group. No serious adverse events of AV block were reported in the placebo group. This finding will be described in the final product label.

8.4.3. Dropouts and/or Discontinuations Due to Adverse Effects

In placebo-controlled study 004, a total of 7 (4.7%) patients in the patisiran-LNP group and 11 patients (14.3%) in the placebo group reported AEs that led to treatment discontinuation. AEs leading to treatment discontinuation reported in 2 or more patients were cardiac failure (2 patients, 1.4%) in the patisiran-LNP group and acute kidney injury (2 patients, 2.6%) in the placebo group. In both treatment groups, all other AEs leading to treatment discontinuation were reported in 1 patient each, as shown in the table in Appendix 13.6.

In all studies pooled together, a total of 13 (6.0%) patients reported an AE leading to discontinuation of patisiran-LNP and study withdrawal. The only AE leading to discontinuation of patisiran-LNP and study withdrawal reported in 2 or more patients was cardiac failure (2 patients, 0.9%) as noted above in Study 004. All other AEs leading to treatment discontinuation were reported in 1 patient by preferred term, as shown in the table in Appendix 13.7.

8.4.4. Significant Adverse Events

Adverse events that occurred during the studies were assessed by the Investigators for severity (mild, moderate, or severe). A subject that experienced the same event more than once was counted only once and at the highest severity for that event.

In Study 004, 28.4% of subjects in the patisiran group had severe AEs compared to 36.4% in the placebo group. There were fewer mild and moderate cardiac disorders in the patisiran group (11.5% and 8.1%, respectively) compared to placebo (18.2% and 10.4%, respectively), but there were more severe cardiac disorders in the patisiran group (8.8%) than in the placebo group (7.8%) as seen in the table in Appendix 13.8. *See the discussion of cardiac deaths in Section 8.5.7.*

Severe AEs in the overall pooled experience with ≥2% of patients included diarrhea (6.4%) and cardiac failure (2.8%).

8.4.5. Treatment Emergent Adverse Events and Adverse Reactions

Results of the FDA analysis of TEAEs are shown in the following table. The most common AEs in the patisiran group that occurred in at least 10% of patients and were more frequent than

placebo were “URI, rhinitis, respiratory tract infection,” infusion reaction, fatigue, and dyspnea. A listing of AEs by body system is shown in the table in Appendix 13.9.

Infusion-related reaction symptoms include arthralgia or pain (including back, neck, or musculoskeletal pain), flushing (including erythema of face or skin warm), nausea, abdominal pain, dyspnea or cough, chest discomfort or chest pain, headache, rash, chills, dizziness, fatigue, increased heart rate or palpitations, hypotension, hypertension, and facial edema.

Table 65: Adverse Reactions from the Placebo-Controlled Trial that Occurred in at Least 5% of ONPATTRO-treated Patients and at Least 3% More Frequently than in Placebo-treated Patients

Adverse Reaction	ONPATTRO N=148 %	Placebo N=77 %
Upper respiratory tract infections [a]	29	21
Infusion-related reaction [b]	19	9
Dyspepsia	8	4
Dyspnea [c, d]	8	0
Muscle spasms [c]	8	1
Arthralgia [c]	7	0
Erythema [c]	7	3
Bronchitis [e]	7	3
Vertigo	5	1

[a] Includes nasopharyngitis, upper respiratory tract infection, respiratory tract infection, pharyngitis, rhinitis, sinusitis, viral upper respiratory tract infection, upper respiratory tract congestion.
[b] Infusion-related reaction symptoms include, but are not limited to: arthralgia or pain (including back, neck, or musculoskeletal pain), flushing (including erythema of face or skin warm), nausea, abdominal pain, dyspnea or cough,
chest discomfort or chest pain, headache, rash, chills, dizziness, fatigue, increased heart rate or palpitations, hypotension, hypertension, facial edema.
[c] Not part of an infusion-related reaction.
[d] Includes dyspnea and exertional dyspnea.
[e] Includes bronchitis, bronchiolitis, bronchitis viral, lower respiratory tract infection, lung infection.

8.4.6. Laboratory Findings

Laboratory findings for patients who received patisiran in the placebo-controlled study (Study 004) and for each long-term open-label study (Studies 003 and Study 006) are summarized. The evaluations included hematology, renal function, liver function tests (LFTs), blood chemistry, thyroid function, and coagulation parameters.

Hematology

Post-baseline abnormalities in hematology laboratory parameters are summarized in the following table, copied from the submission.

Table 66: Summary of Individual Post-Baseline Abnormalities in Hematology Laboratory Parameters (ALN-TTR02-004 Safety Population). Source: Summary of Clinical Safety, p. 102

Parameter	Criterion	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Hemoglobin (g/L)	<100	3 (3.9)	4 (2.7)
Lymphocytes ($\times10^9$/L)	<0.8	15 (19.5)	36 (24.3)
	<0.5	2 (2.6)	8 (5.4)
	>12	0	0
Neutrophils ($\times10^9$/L)	<1.5	2 (2.6)	0
	<1.0	1 (1.3)	0
	≥12	0	2 (1.4)
Platelet Count ($\times10^9$/L)	<75	0	1 (0.7)
	<50	0	0
	≥600	0	0
WBC ($\times10^9$/L)	<3.0	1 (1.3)	3 (2.0)
	≥16	0	1 (0.7)

Abbreviations: WBC=white blood cell.

The averages of the maximum and minimum changes, respectively, from baseline in hematologic parameters are presented in the following tables, generated from the submitted data.

Table 67: Mean Maximum Change from Baseline in the Controlled Study 004. Source: FDA Analysis

Parameter	Placebo	Patisiran 0.3 mg/kg
Platelets	1.3	15
Hemoglobin	-2.6	-1.5
Hematocrit	-0.5	-0.3
Lymphocytes	-0.1	-0.1
Monocytes	-0.07	0.02
Neutrophils	0.9	0.9
Leukocytes	0.4	0.5

Table 68: Mean Minimum Change from Baseline in the Controlled Study 004. Source: FDA Analysis

Parameter	Placebo	Patisiran 0.3 mg/kg
Platelets	-23	-11
Hemoglobin	-11	-8
Hematocrit	-3.2	-2.5
Lymphocytes	-0.5	-0.5
Monocytes	-0.2	-0.1
Neutrophils	-0.6	-0.2
Leukocytes	-1	-0.6

<u>Platelets</u>

In Study 004, no patients had platelet counts <50 $x10^9$/L. The lowest value was 72 x 10^9/L in patisiran subject [redacted] (b) (6) on study day 546. This subject also had a low baseline value of 73 $x10^9$/L. One patient in the patisiran-LNP group had a transient mild AE of thrombocytopenia. In the placebo group, 1 patient had a transient moderate AE of thrombocytopenia and 1 patient had a mild AE of platelet count decreased.

Changes in platelets were similar between the two groups. The elevated mean maximum change in the patisiran group was driven by the following outlier.

The largest outlier in the patisiran group, subject [redacted] (b) (6), had an increase in platelet count of 299 x 10^9 per liter (Baseline = 213 x 10^9 per liter, Study Day 546 = 512 x 10^9 per liter). Review of the subject narrative shows that this subject had an SAE of systemic inflammatory response syndrome and was hospitalized due to pneumonia at the time of this abnormal platelet level. This patient likely had a reactive thrombocytosis due to the infection.

Hemoglobin and Hematocrit

Changes in hemoglobin and hematocrit were similar between the two groups, as seen in the tables above generated from the submitted data.

Lymphocytes

A total of 10 patients (2 [2.6%] in the placebo group and 8 [5.4%] in the patisiran-LNP group) had at least 1 low lymphocyte count <0.5 x10^9/L. These changes may be related to the administration of corticosteroids prior to testing.

The largest outlier in the patisiran group, subject [redacted] (b) (6), had an increase in lymphocyte count of 3.7 x 10^9 per liter (Baseline = 0.7 x 10^9 per liter (low), Study Day 252 = 4.4 x 10^9 per liter (high)). Review of the subject's data finds no report of an adverse event at the time of this lab value. The subject's lymphocyte level returned to normal (2.0 x 10^9 per liter) at the third and final evaluation on Study Day 546.

Neutrophils

The largest negative outlier in the patisiran group, subject [redacted] (b) (6), had a decrease in neutrophil count of 8.5 x 10^9/L (Baseline = 11.1 x 10^9/L (high), Study Day 546 = 2.6 x 10^9/L). However, the baseline level had been high and the remaining measurements during the study were in the normal range.

The largest positive outlier in the patisiran group, subject [redacted] (b) (6), had an increase in neutrophil count of 11.8 x 10^9/L (Baseline = 3.8 x 10^9/L, Study Day 546 = 15.6 x 10^9/L (high)). This subject experienced SAEs of infected skin ulcers (Study Day 554), suggesting that a developing infection was likely the cause of this high neutrophil count.

Leukocytes

The largest negative outlier in the patisiran group, subject [redacted] (b) (6), had a decrease in leukocyte count of 8.4 x 10^9/L (Baseline = 12.9 x 10^9/L (high), Study Day 546 = 4.5 x 10^9/L). However, the baseline level had been high and the remaining measurements during the study were in the normal range.

The second largest negative outlier in the patisiran group, subject [redacted] (b) (6), had a decrease in leukocyte count of 8.3 x 10^9/L (Baseline = 11.8 x 10^9/L (high), Study Day 252 = 3.5 x 10^9/L (low)). However, the baseline level had been high. The second measurement on Study Day 252 was

low and may have been related to the subject's AE of malnutrition noted as unresolved on Study Day 154. The final measurement on Study Day 546 was normal (8 x 10^9/L).

The largest positive outlier in the patisiran group, subject [redacted] (b) (6), had an increase in leukocyte count of 12.3 x 10^9/L (Baseline = 5 x 10^9/L, Study Day 546 = 17.3 x 10^9/L (high)). This subject experienced SAEs of infected skin ulcers (Study Day 554), suggesting that a developing infection was likely the cause of this high leukocyte count.

There does not appear to be a clinically significant difference in hematologic parameters between the drug and placebo groups. Note that both patisiran and placebo groups received corticosteroids as part of the premedication regimen intended to limit infusion related reactions. Corticosteroids may reduce the levels of lymphocytes and monocytes and increase the levels of neutrophils and WBCs.

Renal Function

The following table summarizes the worst post-baseline estimated glomerular filtration rates (eGFR) for Study 004.

Table 69: Worst post-baseline eGFR by actual treatment – All Patients. Source: FDA Analysis

GFR_Post_Base_Cat	PLACEBO	Patisiran 0.3 mg/kg
Stage1: eGFR >=90	36 (46.8%)	70 (47.3%)
Stage2: eGFR 60-89	27 (35.1%)	46 (31.1%)
Stage3a: eGFR 30-44	1 (1.3%)	1 (0.7%)
Stage3b: eGFR 45-59	9 (11.7%)	18 (12.2%)
Stage4: eGFR 15-29	3 (3.9%)	5 (3.4%)
Stage5: eGFR <15	0 (0.0%)	5 (3.4%)

As seen in the above table, there were 5 patients ([redacted] (b) (6), and [redacted] (b) (6)) in the patisiran group who had transient post-baseline shifts to <15 mL/min/1.73 m^2 with concurrent creatinine values >3 × baseline or >4 mg/dL compared with none in the placebo group. Two of the 5 subjects ([redacted] (b) (6)) had eGFR below 60 at baseline, suggesting underlying renal disease. All of the subjects were on at least one concomitant medication with a potential renal adverse effect. All of the low eGFR measurements were transient and returned to the subjects' baseline ranges despite continued treatment with patisiran. There were no Adverse Events associated with these abnormal eGFR values. There

were no significant weight changes at the times of the abnormal eGFRs. Kidney disease was not listed in the medical history of any of these 5 subjects. The following table summarizes the data from these 5 subjects.

Table 70: Subjects in the Patisiran Group of Study 004 with Transient Post-Baseline Shifts to eGFR <15 mL/min/1.73 m^2. Concomitant medications in red italics have potential renal adverse effects.

Subject	Visit Day	Days Since Last Dose	eGFR (mL/min/1.73 m^2)	Concomitant Medications	Recent Weights (kg)	Medical History
(b) (6) [redacted]	84	21	12.8	rivaroxaban, pregabalin, furosemide, vitamin A, *pantoprazole*	Day 63: 69; Day 84: 68.6	erectile dysfunction, atrial fibrillation, paresthesia
	189	21	13.6	Florinef, pregabalin, loperamide, *Omeprazole*, amitriptyline, protein supplement, *ciproflox*, caltrate, furosemide	Day 168: 55.3; Day 189: 56	neuropathic pain, postural hypotension, diarrhea
	357	21	4.9	fosfomycin, *ciprofloxacin*, loperamide, caltrate, sennoside, *paracetamol*, amoxicillin and *clavulanate*, benzonatate	Day 336: 58.2 Day 357: 57.8	polyneuropathy
	189	21	13.4	same as above	Day 168: 59.7 Day 189: 58.5	

[redacted] (b) (6)	357	21	13.7	loperamide, *paracetamol*, pregabalin, caltrate	Day 336: 50.7; Day 357: 51.2	diarrhea, neuropathy, subconjunctival amyloid
	189	21	14.5	pregabalin, loperamide, *paracetamol*, *ibuprofen*, caltrate, *tobramycin*, dexamethasone, amoxicillin, *clavulanate*	Day 168: 50.1; Day 189: 51.4	neuropathic pain, diarrhea

The averages of the maximum and minimum changes, respectively, from baseline in renal parameters are presented in the following tables, generated from the submitted data.

Table 71: Mean Maximum Change from Baseline in the Controlled Study 004. Source: FDA Analysis

Parameter	Placebo	Patisiran 0.3 mg/kg
Blood Urea Nitrogen (BUN)	0.8	1.4
Creatinine	12	27
Creatinine Clearance	18	17

Table 72: Mean Minimum Change from Baseline in the Controlled Study 004. Source: FDA Analysis

Parameter	Placebo	Patisiran 0.3 mg/kg
Blood Urea Nitrogen (BUN)	-1.2	-1.3
Creatinine	-8.2	-7.6
Creatinine Clearance	-12	-22

The largest positive BUN outlier in the patisiran group, subject [redacted] (b) (6), had an increase in BUN of 24.6 mmol/L (Baseline = 12.5 mmol/L (high), Study Day 462 = 37.1 mmol/L (high)). However, the baseline level had been high and remained elevated throughout the study. This subject's eGFR was also abnormally low at baseline (37.6) and throughout the study, indicating kidney disease at baseline.

The largest positive creatinine outlier in the patisiran group, subject [redacted] (b) (6), had an increase in serum creatinine of 637 micromol/L (Baseline = 97 micromol/L (high), Study Day 357 = 734 micromol/L (high)). However, the baseline level had been high and remained elevated throughout the study. This subject's eGFR was also abnormally low at baseline (42) and throughout the study, indicating kidney disease at baseline. This subject also had multiple urinary tract infections during the study which may have further impaired renal function.

Renal function in Study 003 is summarized in the following table, copied from the submission. There was one subject in Study 003 with a decline in eGFR from baseline to a low range of 30-59. In Study 006, there were 2 patients, 1 in the 004 placebo group and 1 in the 004 patisiran-LNP group who had an eGFR of 15 to 29 mL/min/1.73 m^2 at baseline in Study 006 that shifted to <15 mL/min/1.73 m^2. At the time of the shift, both patients had concurrent creatinine values >3 × baseline or >4 mg/dL. Both patients had a history of renal impairment and hATTR amyloidosis. In 1 patient, the shift occurred in the setting of worsening of congestive heart failure. Both patients discontinued study drug due to worsening of renal impairment.

Individual patient summaries are listed in Appendix 13.10.

Table 73: Shift from Baseline eGFR for Study 003. Source: Study 003 CSR.

Shift from Baseline to Worst Post-Baseline for eGFR (Safety Analysis Set)

Baseline eGFR		Worst Post-Baseline						
Categories	Statistic	>=90	60-89	30-59	15-29	<15	Missing	Total
>=90	n(%)	12(44.4)	5(18.5)	0	0	0	0	17(63.0)
60-89	n(%)	0	7(25.9)	1(3.7)	1(3.7)	0	0	9(33.3)
30-59	n(%)	0	0	1(3.7)	0	0	0	1(3.7)
15-29	n(%)	0	0	0	0	0	0	0
<15	n(%)	0	0	0	0	0	0	0
Missing	n(%)	0	0	0	0	0	0	0
Total	n(%)	12(44.4)	12(44.4)	2(7.4)	1(3.7)	0	0	27(100.0)

Reviewer Comment: Renal impairment is a known complication of hATTR amyloidosis. Outliers in the patisiran group generally had abnormal baseline values, renal comorbidities, or concomitant medications with known potential renal adverse effects. There does not appear to be a clinically significant difference in renal function change between the drug and placebo groups. The Division of Cardiovascular and Renal Products was consulted regarding the five subjects in the patisiran group with transient eGFR <15 mL/min/1.73m^2 and concluded that in the absence of any nonclinical or clinical signals of renal safety, as evaluated by DNP, that these cases were very difficult to interpret and plausibly likely to be unrelated to treatment with

patisiran.

Liver Function Test Parameters

The following tables show the averages of the maximum and minimum changes, respectively, from baseline in liver function test parameters for Study 004.

Table 74: Mean Maximum Change from Baseline in the Controlled Study 004. Source: FDA Analysis

Parameter	Placebo	Patisiran 0.3 mg/kg
Bilirubin	3	3
AST	7	13
ALT	7	14
Alkaline phosphatase	14	16

This table shows higher mean maximum change from baseline of AST and ALT in the patisiran group compared to placebo. This finding is discussed further below.

Table 75: Mean Minimum Change from Baseline in the Controlled Study 004. Source: FDA Analysis

Parameter	Placebo	Patisiran 0.3 mg/kg
Bilirubin	-1	-2
AST	-4	-3
ALT	-7	-6
Alkaline phosphatase	-12	-10

<u>Bilirubin</u>

The following table, generated from the submitted data, shows that the percentage of subjects in the patisiran group with abnormal elevations of bilirubin from a normal baseline (~3%) was similar to the percentage of subjects in the placebo group (~4%).

Table 76: Shift Table of Bilirubin Elevations Relative to Baseline in Study 004. Source: FDA Analysis

	Patisiran 0.3 mg/kg		PLACEBO		Subjects(filtered)
BILI_Post_Base_Category	Base BILI Normal	Base BILI > ULN <=2xULN	Base BILI Normal	Base BILI > ULN <=2xULN	
BILI Normal	131 (88.5%)	2 (1.4%)	70 (90.9%)	1 (1.3%)	204 (90.7%)
BILI > ULN <=2xULN	5 (3.4%)	5 (3.4%)	3 (3.9%)	0 (0.0%)	13 (5.8%)
Subjects(filtered)	136 (91.9%)	7 (4.7%)	73 (94.8%)	1 (1.3%)	225 (100.0%)
1stColItemSubjects	148 (100.0%)	148 (100.0%)	77 (100.0%)	77 (100.0%)	(Denom=1stColTot)

Aspartate Aminotransferase

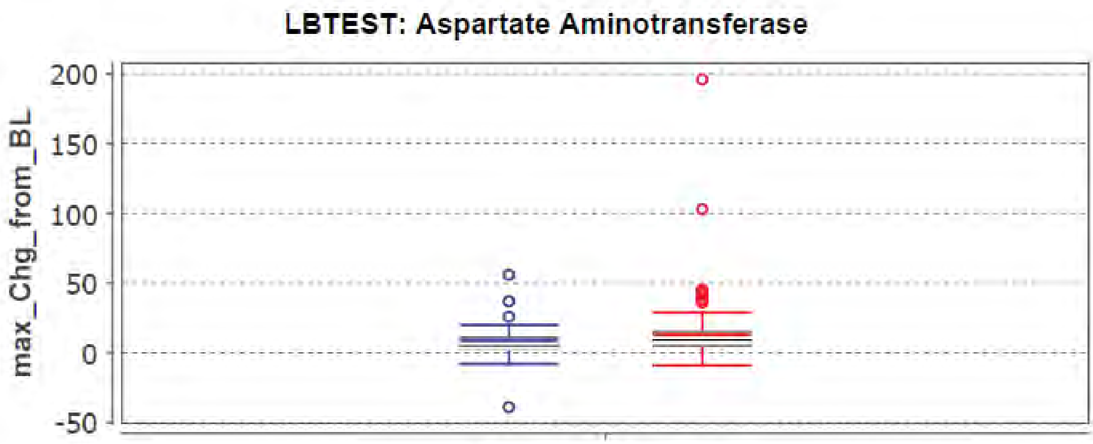
The following table, generated from the submitted data, shows that more subjects in the patisiran group had abnormal elevations of AST from a normal baseline (~18%) than subjects in the placebo group (~5%).

Table 77: Shift Table of AST Elevations Relative to Baseline in Study 004. Source: FDA Analysis

	Patisiran 0.3 mg/kg		PLACEBO		Subjects(Filtered)
AST_Post_Base_Category	Base AST Normal	Base AST > ULN <=2xULN	Base AST Normal	Base AST > ULN <=2xULN	
AST Normal	114 (77.0%)	0 (0.0%)	71 (92.2%)	1 (1.3%)	186 (82.7%)
AST > ULN <=2xULN	26 (17.6%)	4 (2.7%)	4 (5.2%)	0 (0.0%)	34 (15.1%)
AST > 2xULN <=3xULN	1 (0.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)
Subjects(Filtered)	141 (95.3%)	4 (2.7%)	75 (97.4%)	1 (1.3%)	225 (100.0%)
1stColItemSubjects	148 (100.0%)	148 (100.0%)	77 (100.0%)	77 (100.0%)	(Denom=1stColTot)

However, these changes were generally transient, small, and of similar magnitude between the patisiran and placebo groups.

Figure 11: Aspartate Aminotransferase: Maximum Change from Baseline in Study 004. Plot shows median, quartiles, and outliers.



ALN-TTR02-004

	PLACEBO	Patisiran 0.3 mg/kg
Mean	6.805	12.973
Median	5.000	9.000
Q1	3.000	5.000
Q3	11.000	15.000
Min	-39.000	-9.000
Max	56.000	196.000
N	77	148

STUDYID

Description of Actual Arm

PLACEBO Patisiran 0.3 mg/kg

The largest positive outlier in the patisiran group, subject [redacted] (b) (6), had normal AST throughout the study with a minimum AST of 14 U/L on Study Day 0. However, this subject had a high AST at initial Screening (Study Day -15) of 210 U/L, leading to a large change of 196 U/L.

A second screening test yielded a normal AST of 16 U/L on Study Day -8, suggesting that the first high level may have been a laboratory error.

Alanine Aminotransferase

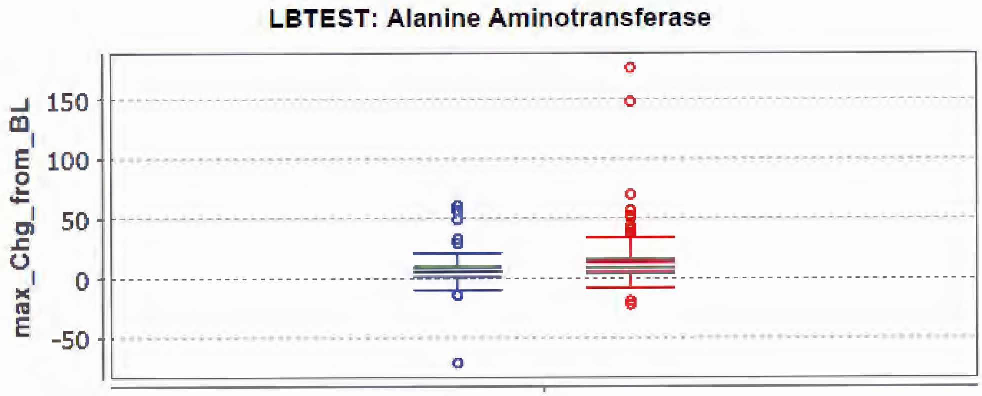
The following table, generated from the submitted data, shows that more subjects in the patisiran group had abnormal elevations of ALT from a normal baseline (~20%) than subjects in the placebo group (~10%).

Table 71: Shift Table of ALT Elevations Relative to Baseline in Study 004. Source: FDA Analysis

	Patisiran 0.3 mg/kg			PLACEBO			Subjects(filtered)
ALT_Post_Base_Category	Base ALT Normal	Base ALT > ULN <=2xULN	Base ALT > 2xULN <=3xULN	Base ALT Normal	Base ALT > ULN <=2xULN	Base ALT > 2xULN <=3xULN	
ALT Normal	101 (68.2%)	4 (2.7%)	0 (0.0%)	62 (80.5%)	3 (3.9%)	1 (1.3%)	171 (76.0%)
ALT > ULN <=2xULN	26 (17.6%)	7 (4.7%)	1 (0.7%)	8 (10.4%)	1 (1.3%)	0 (0.0%)	43 (19.1%)
ALT > 2xULN <=3xULN	2 (1.4%)	3 (2.0%)	0 (0.0%)	0 (0.0%)	1 (1.3%)	0 (0.0%)	6 (2.7%)
ALT > 3xULN <=5xULN	1 (0.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)
Subjects(filtered)	130 (87.8%)	14 (9.5%)	1 (0.7%)	70 (90.9%)	5 (6.5%)	1 (1.3%)	225 (100.0%)
1stColItemSubjects	148 (100.0%)	148 (100.0%)	148 (100.0%)	77 (100.0%)	77 (100.0%)	77 (100.0%)	(Denom=1stColTot)

However, these changes were generally transient, small, and of similar magnitude between the patisiran and placebo groups.

Figure 12: Alanine Aminotransferase: Maximum Change from Baseline in Study 004. Plot shows median, quartiles, and outliers.



ALN-TTR02-004	PLACEBO	Patisiran 0.3 mg/kg
Mean	7.091	13.520
Median	5.000	9.000
Q1	1.000	4.000
Q3	10.000	16.000
Min	-71.000	-22.000
Max	61.000	175.000
N	77	148

STUDYID

Description of Actual Arm

■ PLACEBO ■ Patisiran 0.3 mg/kg

The largest positive outlier in the patisiran group, subject (b) (6), is described above under AST. This large change was due to a high level at initial screening that appears to have been a laboratory error, given that there was a normal repeated test.

The second largest positive outlier in the patisiran group, subject (b) (6), had an increase in ALT of 151 U/L (Baseline = 16 U/L, Study Day 84 = 167 U/L (high)) while AST and bilirubin levels remained normal. The subject had an AE of diarrhea on Study Day 82. The ALT was again normal on Study Day 105 (35 U/L).

The changes in bilirubin level, AST, and ALT are similar for the patisiran and placebo groups with no clinically significant differences. Note that hepatomegaly with elevation of liver enzymes is common in systemic amyloidosis. There were no cases of liver enzyme elevation that were consistent with Hy's Law (ALT or AST ≥ 3X ULN and bilirubin > 2X ULN).

Blood Chemistry

Post-baseline abnormalities in blood chemistry laboratory parameters are summarized in the following table, copied from the submission.

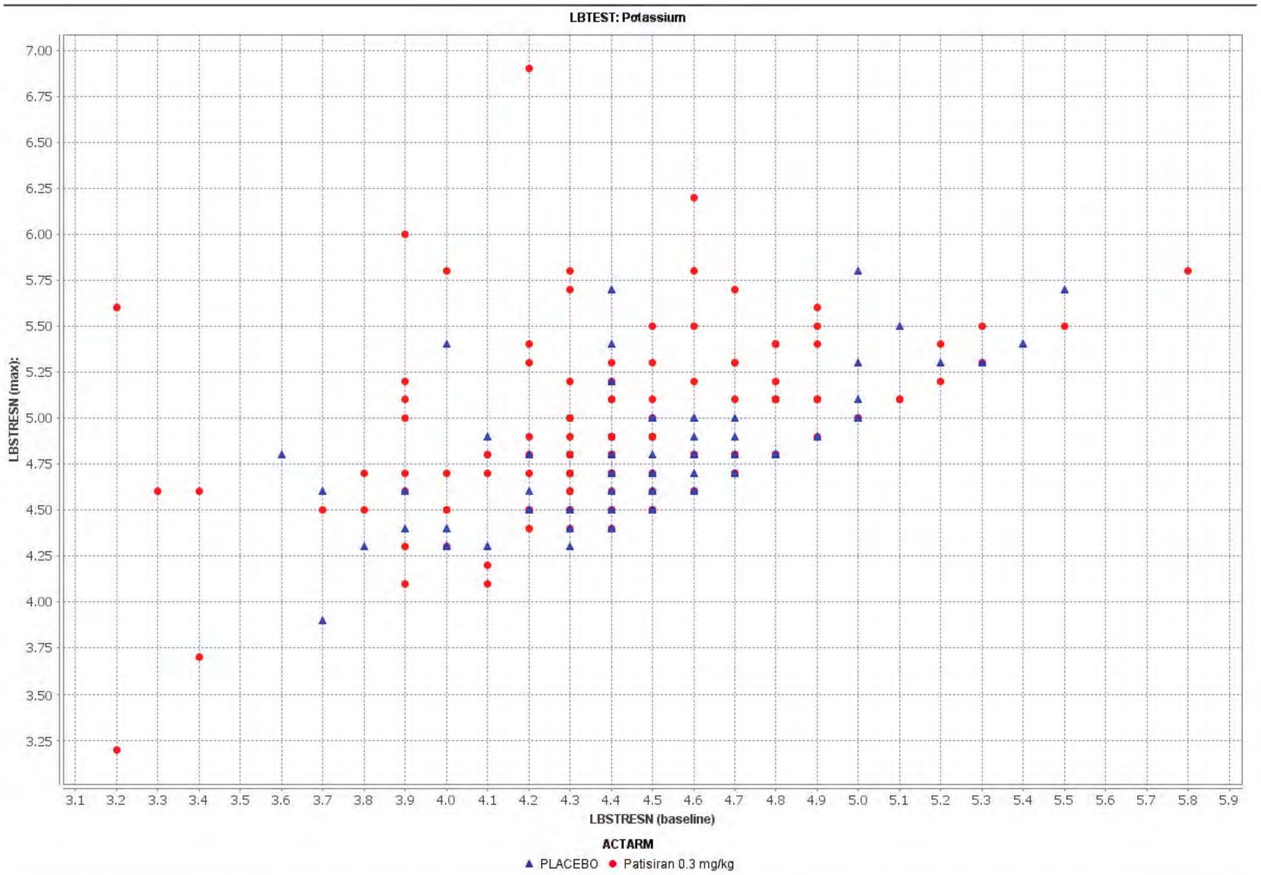
Table 78: Summary of Individual Post-Baseline Abnormalities in Blood Chemistry Laboratory Test Results (ALN-TTR02-004 Safety Population). Source: Summary of Clinical Safety, p. 108

Laboratory Test	Criterion	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Albumin (g/L)	<30	5 (6.5)	5 (3.4)
Calcium (mmol/L)	<2	3 (3.9)	8 (5.4)
	>2.9	0	0
Creatinine (µmol/L)	>2 × baseline	2 (2.6)	10 (6.8)
	>3 × baseline or > 4 mg/dL	1 (1.3)	7 (4.7)
Glucose (mmol/L)	<3.0	1 (1.3)	1 (0.7)
	≥13.9	3 (3.9)	2 (1.4)
Phosphate (mmol/L)	<0.6	3 (3.9)	2 (1.4)
Potassium (mmol/L)	<3.0	2 (2.6)	2 (1.4)
	>6.0	0	2 (1.4)
Sodium (mmol/L)	<130	0	3 (2.0)
	>155	0	0

Note: For creatinine, 4 mg/dL is equivalent to 353.6 µmol/L.

The following figure, generated from the submitted data, shows the maximum potassium values versus baseline for all subjects in Study 004.

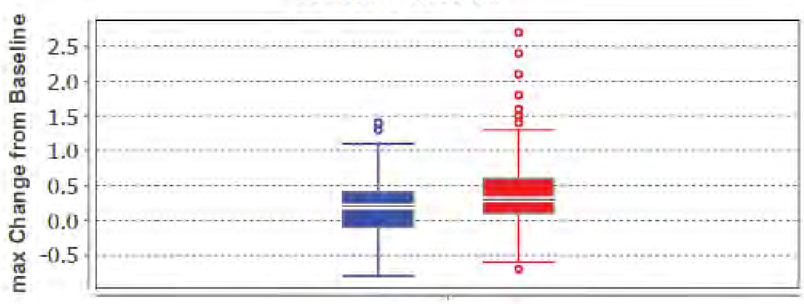
Figure 13: Scatter Plot of Maximum Potassium Values versus Baseline for all Subjects in Study 004. Source: FDA Analysis



The maximum potassium levels have more outliers in the patisiran group. Outliers generally had abnormal baseline values, except for subjects (b) (6) Subjects (b) (6) and (b) (6) had transient hyperkalemia that was not associated with an adverse event. Subject (b) (6) had hyperkalemia in the setting of reduced glomerular filtration rate, discussed above under Renal Function.

The following figure shows the maximum changes from baseline for potassium in Study 004.

Figure 14: Potassium: Maximum Change from Baseline in Study 004. Plot shows median, quartiles, and outliers. Source: FDA Analysis



	ALN-TTR02-004	
	PLACEBO	Patisiran 0.3 mg/kg
Mean	0.1737	0.3897
Median	0.2000	0.3000
Q1	-0.1000	0.1000
Q3	0.4000	0.6000
Min	-0.8000	-0.7000
Max	1.4000	2.7000
N	76	145

STUDYID

ACTARM

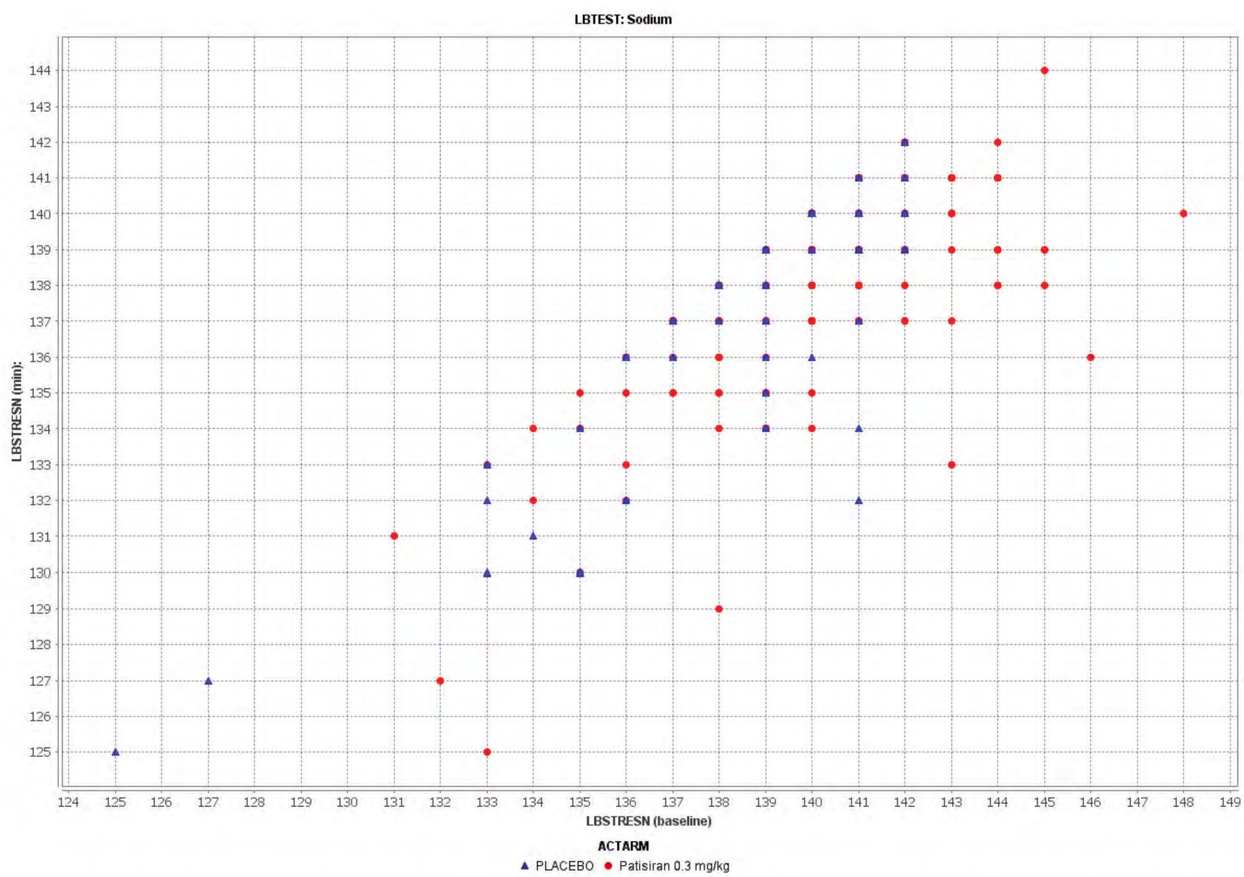
PLACEBO Patisiran 0.3 mg/kg

The largest positive outlier in the patisiran group, subject [redacted] (b) (6), had an increase in potassium of 2.7 mmol/L (Baseline = 4.2 mmol/L, Study Day 85 = 6.9 mmol/L (high)) that was associated with high levels of BUN (15.4 mmol/L) and creatinine (415 micromol/L), as discussed above under Renal Function. There were no adverse events reported at the time of this high potassium level. The potassium level was again normal on Study Day 190 (4.3 mmol/L) despite continued treatment.

The second largest positive outlier in the patisiran group, subject [redacted] (b) (6), had an increase in potassium of 2.4 mmol/L (Baseline = 3.2 mmol/L, Study Day 85 = 5.6 mmol/L (high)) with normal levels of BUN and creatinine. There were no adverse events reported at the time of this high potassium level. The potassium level was normal on Study Day 190 (3.5 mmol/L) despite continued treatment.

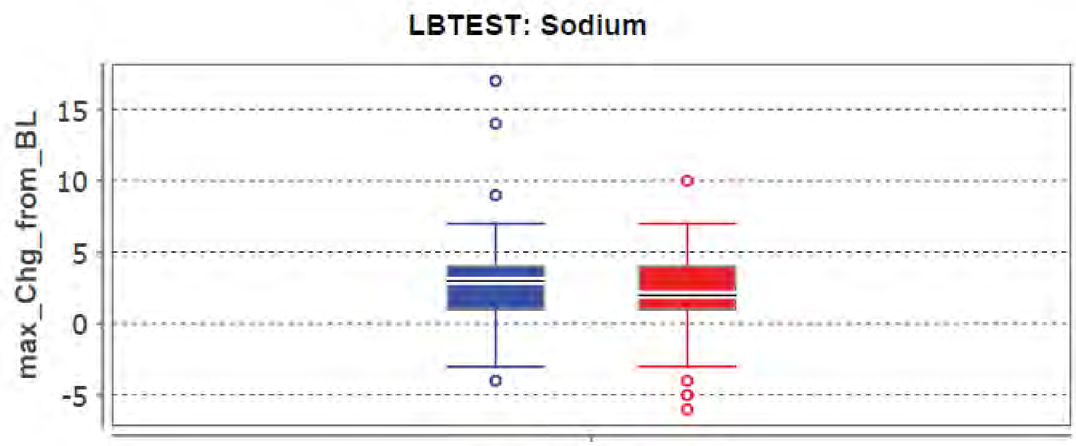
The following figure, generated from the submitted data, shows the minimum sodium values versus baseline for all subjects in Study 004.

Figure 15: Scatter Plot of Minimum Sodium Values versus Baseline for all Subjects in Study 004. Source: FDA Analysis



The minimum sodium levels are similar in the patisiran and placebo groups. Subjects with low values (<133 mEq/L) generally had abnormally low baseline values. Subject [redacted] (b) (6) in the patisiran group had a normal baseline of 138 mEq/L and a low value of 129 on study day 357 associated with congestive heart failure.

Figure 16: Sodium: Maximum Change from Baseline in Study 004. Plot shows median, quartiles, and outliers.



ALN-TTR02-004	PLACEBO	Patisiran 0.3 mg/kg
Mean	3.0000	2.0966
Median	3.0000	2.0000
Q1	1.0000	1.0000
Q3	4.0000	4.0000
Min	-4.0000	-6.0000
Max	17.0000	10.0000
N	76	145

STUDYID

ACTARM

PLACEBO Patisiran 0.3 mg/kg

The largest negative outlier in the patisiran group, subject (b) (6), had a minimum sodium level of 136 mmol/L (normal) on Study Day 356 and a maximum level of 146 mmol/L at baseline. This change is not clinically significant.

There does not appear to be a clinically significant difference in changes of blood chemistry

parameters between the drug and placebo groups.

Thyroid Parameters

As TTR is minor transporter of thyroxine, thyroid parameters were analyzed in the patisiran-LNP studies. In patisiran nonclinical studies, decreases in total thyroxine (up to 50%) were observed.

At Baseline in Study 004, 17 patients (7.6%) had medical history of hypothyroidism: 13 patients (8.8%) in the patisiran-LNP group and 4 patients (5.2%) in the placebo group. During Study 004, an AE of hypothyroidism was reported in 1 patient each in the patisiran-LNP (0.7%) and placebo (1.3%) groups. In the overall pooled analysis including studies 003 and 006, there were 2 patients with hypothyroidism.

Thyroid abnormalities are similar for the patisiran and placebo groups with no clinically significant differences. Note that hypothyroidism is known to occur in systemic amyloidosis due to thyroid infiltration by amyloid deposits.

Coagulation Parameters

In Study 004, the mean coagulation parameters are similar for the patisiran and placebo groups with no clinically significant differences. There is a greater numerical increase in INR above normal in the placebo group at the end of the study (Mean % change = 16% placebo; 6% patisiran). Note that coagulation abnormalities in amyloidosis patients can be due to the interaction of clotting factors with amyloid. Some patients were on anticoagulation therapy during the study.

There were no Grade 3 (severe) or Grade 4 (potentially life threatening) shifts in activated partial thromboplastin time (aPTT) or prothrombin time during the study.
In Study 003, 2 patients had clinically significant elevated coagulation parameter levels (1 patient had clinically significant aPTT and prothrombin International Normalized Ratio (INR) levels and 1 patient had clinically significant prothrombin time, aPTT, and INR levels), as shown in the following table copied from the submission. In Study 006, no patients had prothrombin time values that were Grade 3 or 4.

Table 79: Patients with Abnormal INR > 2 during Study 004. Note that patients could participate in the study while on anticoagulation therapy if their INR was <= 3.5. Source: Study 004 data files

Patient ID	Group	Baseline INR	Highest INR	Study Day
[redacted] (b) (6)	Placebo	3.5	3.5	-21
[redacted]	Placebo	2.6	2.6	-41
[redacted]	Patisiran	2.5	2.5	-23
[redacted]	Placebo	2.3	2.5	557
[redacted]	Placebo	0.9	2.4	547
[redacted]	Placebo	0.9	2.4	547

8.4.7. **Vital Signs**

In the placebo-controlled Study 004, there were *no clinically significant differences in vital signs between the placebo and patisiran-LNP groups except for a decrease in weight in the placebo group*. Vital signs were assessed by plotting time trends of change from baseline over the course of the study. Blood pressure changes were also assessed through scatterplots of baseline versus maximum value. The applicant reported that the mean change in weight from baseline was negative 3.2kg in the placebo group, compared to a mean positive change from baseline of 1.4kg in the patisiran group. Weight loss is a common feature of ATTR amyloidosis and may be due to gastrointestinal autonomic dysfunction and diarrhea (Falk et al., 1997).

The following tables show the averages of the maximum and minimum percent changes, respectively, from baseline in vital signs.

Table 80: Mean Maximum % Change from Baseline in the Controlled Study 004. Source: FDA Analysis

Parameter	Placebo	Patisiran 0.3 mg/kg
Weight	4	7
Systolic Blood Pressure	19	22
Diastolic Blood Pressure	23	24
Heart Rate	21	23

Table 81: Mean Minimum % Change from Baseline in the Controlled Study 004. Source: FDA Analysis

Parameter	Placebo	Patisiran 0.3 mg/kg

Weight	-7	-4
Systolic Blood Pressure	-21	-20
Diastolic Blood Pressure	-23	-24
Heart Rate	-20	-21

8.4.8. **Electrocardiograms (ECGs)**

Computerized 12-lead electrocardiogram (ECG) recordings were read by a cardiologist or qualified physician. In placebo-controlled Study 004, ECGs were collected at baseline and at Weeks 18, 36 to 39, 57, 79 to 80, or at early termination. At the Month 18 visit, there was no clinically meaningful difference in the proportions of ECG abnormalities between the placebo and drug treatment groups. Outliers were assessed by examining baseline versus post-baseline ECG parameters as shown in the figures in Appendix 13.13.

8.4.9. **QT**

Following consultation with the QT Interdisciplinary Review Team, the Agency previously agreed with the applicant on June 16, 2016 that a thorough QTc clinical study was not required to support registration of patisiran. Patisiran has a low likelihood of direct ion channel interactions. There is no evidence from nonclinical or clinical data to suggest that patisiran has the potential to delay ventricular repolarization. ECG monitoring was implemented in Phase 2 and Phase 3 trials. As seen in the ECG shift plots in Appendix 13.13, *there is no clinically significant difference in QT interval changes between the placebo and patisiran groups of Study 004.*

8.4.10. **Immunogenicity**

Anti-drug antibodies (ADA), defined as serum IgG/IgM antibodies specific to PEG2000- C -DMG, were tested at baseline (Day 0) and 5 visits post-baseline (Days 21, 126, 252, 399 and 546). As seen in the following table, copied from the submission, *more patients in the patisiran group (3.4%) developed ADAs than in the placebo group (1.3%).* Five patisiran patients ([redacted] (b) (6)) had negative ADA status at baseline and tested positive for ADA after treatment (Day 21 and/or Day 126). Titer in all of the 5 patients ranged from 40 to 80. These 5 patients tested positive for ADA on Day 21 and/or Day 126 but tested negative in subsequent visits until end of the study (Day 252, 399, and 546). Review of the AEs of these ADA-positive subjects did

not suggest an association of ADA positivity with any particular AE.

Table 82: Summary of Patients with ADA Results (Safety Population). Source: Study 004 CSR.

	Placebo N=77	Patisiran-LNP N=148
Patients with ADA measurement at baseline, N	77	147[a]
Patients that were ADA positive at baseline, N (%)	1 (1.3)[b]	1 (0.7)[b]
Patients with at least 1 postdose ADA measurement	77	146[c]
Patients that were ADA positive postdose, N (%)	1 (1.3)[d]	6 (4.1)[d]
Patients with a baseline and at least 1 postdose ADA measurement	77	145[e]
Patients with treatment-emergent ADA, N (%)	1 (1.3)	5 (3.4)

Baseline is defined as Day 0 predose.

Treatment-emergent ADA is defined as number of patients that were ADA positive postdose over number of patients with a baseline and at least 1 postdose ADA measurement.

[a] One patient [redacted] (b) (6) in the patisiran-LNP group had a missing ADA measurement at baseline (Day 0 predose).

[b] Two patients tested ADA positive at baseline as follows: In the patisiran-LNP group patient [redacted] (b) (6) tested positive for ADA at baseline (titer=40) and on Day 126 (titer = 40); his patient's ADA status was not treatment induced or boosted. In the placebo group, patient [redacted] (b) (6) tested positive for ADA at baseline (titer=40) and was ADA negative post-treatment for all visits.

[c] Two patients in the patisiran-LNP group ([redacted] (b) (6) and [redacted] (b) (6)) had missing postdose ADA measurements, but had baseline measurements available.

[d] In the patisiran-LNP group patients [redacted] (b) (6) tested positive for ADA postdose. In the placebo group, patient [redacted] (b) (6) tested positive for ADA postdose.

[e] In the patisiran-LNP group, 2 patients ([redacted] (b) (6) and [redacted] (b) (6)) had missing postdose ADA measurements, but had baseline measurement available; 1 patient [redacted] (b) (6)) had missing baseline ADA measurement, but had postdose ADA measurements available.

8.5. Analysis of Submission-Specific Safety Issues

8.5.1. Adverse Events Related to Premedications

During the clinical development program of patisiran, two premedication regimens were used to reduce the chance of infusion related reactions: the original premedication regimen and later a reduced premedication regimen. These regimens are described in detail in Section 7.1.4. Both regimens included corticosteroids, H1/H2 blockers, and paracetamol or equivalents. Investigators were asked to assess whether an AE was related to premedications or to a study procedure.

The original premedication regimen was used in the 2 healthy volunteer studies (001 and 005) and in the beginning of studies 003, 004, and 006. The switch to the reduced premedication regimen was made after some patients in Study 003 experienced AEs considered possibly related to premedication. In Study 003, sixteen patients (59.3%) had AEs related to premedications, including flushing (7 patients, 25.9%), and insomnia (3 patients, 11%). Two patients had SAEs related to the steroid premedication; 1 patient had a foot abscess and osteomyelitis, and 1 patient with worsening osteopenia had femur and tibia fractures.

This reviewer agrees that the foot abscess, osteomyelitis, osteopenia, and bone fractures were likely related to the steroid premedication. The rates of infection in the placebo and patisiran groups were similar.

Examples of the premedications used in Study 004 include diphenhydramine, paracetamol, dexamethasone, and ranitidine. The applicant's full list of premedication-related adverse events is copied in Appendix 13.11. Adverse events related to premedication (in >=3% of patients in either the patisiran or placebo group) included osteoporosis, dizziness, somnolence and insomnia. Clinical investigators reported SAEs related to premedications in 4 patients: 1 patient with deafness unilateral in the patisiran-LNP group; and 3 patients in the placebo group (1 patient with esophagitis, pulmonary edema and urinary retention; 1 patient with urinary tract infections; and 1 patient with erysipelas and skin ulcer).

Reviewer Comment: The multiple premedications used make it difficult to assess which AEs are

related to which premedications, or if they are actually related to patisiran. However, osteoporosis, dizziness, and insomnia are known reactions to corticosteroids such as dexamethasone. Somnolence is a known reaction to some antihistamines.

8.5.2. **Infusion Related Reactions**

Infusion-related reactions (IRRs) are a known side effect of drugs administered as LNP infusions. In order to decrease the risk of potentially life-threatening hypersensitivity reactions, such as anaphylaxis, the previously described premedication regimen was given to all study participants. For both the placebo-controlled Study 004 and for the overall pooled clinical studies of patisiran, there have been no SAEs of anaphylaxis, anaphylactoid, or severe hypersensitivity reactions. One SAE of syncope and hypotension during patisiran infusion in the expanded access program is described below.

The premedication regimen did not completely prevent some IRRs from occurring, as shown in the following table copied from the submission. For Study 004, the following infusion related reaction signs and symptoms were reported in at least 2% of patients in the patisiran group: back pain (6.1%), flushing (4.1%), nausea (3.4%), headache (2.7%), and arthralgia and dyspnea (2.0% each). The only IRR sign or symptom reported in at least 2% of patients in the placebo group was flushing (7.8%) (Summary of Clinical Safety, p. 74). One patient ([redacted] (b) (6)) had the infusion discontinued and withdrew from the study due to flushing that resolved after 15 minutes without treatment. In the overall pooled data of all patisiran studies, IRRs were reported in 22.0% of patients, including flushing (6.0%), back pain (5.5%), nausea (3.7%), dyspnea (2.8%), and headache (2.3%) (Summary of Clinical Safety, p. 80).

Table 83: Infusion-Related Reactions Signs and Symptoms in 2 or More Patients in Any Group (ALN-TTR02-004 Safety Population). Source: Summary of Clinical Safety, p. 75

System Organ Class/ Preferred Term	Number of Patients (%)[a]/Events[b]	
	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Number of patients with at least 1 IRR and number of IRRs (AEs)	7 (9.1) /79	28 (18.9) /145
Number of IRR symptoms (events)	105	223
Gastrointestinal disorders	0	9 (6.1) /15
Abdominal pain	0	2 (1.4) /2
Abdominal pain lower	0	2 (1.4) /2
Abdominal pain upper	0	2 (1.4) /4
Nausea	0	5 (3.4) /5
General disorders and administration site conditions	2 (2.6) /25	10 (6.8) /46

System Organ Class/ Preferred Term	Number of Patients (%)[a]/Events[b]	
	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Chest discomfort	0	2 (1.4) /6
Chest pain	0	2 (1.4) /8
Chills	1 (1.3) /1	2 (1.4) /10
Fatigue	0	2 (1.4) /9
Injection site erythema	0	2 (1.4) /2
Injection site swelling	0	2 (1.4) /2
Pain	0	2 (1.4) /6
Musculoskeletal and connective tissue disorders	1 (1.3) /2	9 (6.1) /74
Arthralgia	0	3 (2.0) /8
Back pain	0	9 (6.1) /52
Nervous system disorders	1 (1.3) /1	7 (4.7) /11
Headache	1 (1.3) /1	4 (2.7) /7
Respiratory, thoracic and mediastinal disorders	0	5 (3.4) /11
Cough	0	2 (1.4) /2
Dyspnea	0	3 (2.0) /3
Skin and subcutaneous tissue disorders	1 (1.3) /14	5 (3.4) /16
Skin warm	0	2 (1.4) /2
Vascular disorders	6 (7.8) /36	9 (6.1) /40
Flushing	6 (7.8) /36	6 (4.1) /34
Hypotension	0	2 (1.4) /4

Abbreviations: AE=adverse event; IRR=infusion-related reaction; PT=preferred term; SOC=system organ class.
a. If a patient experienced more than 1 event in a given SOC, that patient is counted once for the SOC. If a patient experienced more than 1 event with a given PT, that patient is counted only once for that PT. Percentages are based out of the total number of patients (N) who were on study at the start of the indicated exposure duration category.
b. The total number of events for all patients; a patient can be counted more than once if the patient has multiple events.
Source: CSR ALN-TTR02-004, Table 14.3.1.17.1

There was no increase in IRRs when patients were on the reduced premedication regimen (patisiran (7%), placebo (5%)) compared to the original regimen (patisiran (18%), placebo (10%)) (Summary of Clinical Safety, p. 79).

The following SAE IRR reports were received after the original NDA submission.

Patient [redacted] (b) (6) in open-label study 007 had a history of chronic intermittent abdominal pain due to mesenteric ischemia and experienced severe abdominal pain following patisiran infusion which resolved after treatment with ondansetron, morphine, and hydromorphone.

Patient [redacted] (b) (6), a 71-year-old male with cardiac amyloidosis and polyneuropathy in the expanded access program, had an SAE IRR of syncope despite premedication with 10mg dexamethasone, as described in the following history copied from the safety report. The report does not state if he also received acetaminophen and H1/H2 blockers as premedication.

“5 minutes after the start of the second infusion of patisiran (ALN-TTR02), the patient began to experience an infusion related reaction. Symptoms included warm sensation, flushing, tachycardia, collapse and sudden loss of consciousness while sitting in his wheelchair. The patient wasn’t under cardiovascular monitoring at the time of the event however blood pressure was not palpable on the radial and carotid arteries. The patisiran (ALN-TTR02) infusion was stopped and the patient was given intravenous prednisone, Sterofundin and noradrenaline. Following treatment, the patient’s symptoms declined, skin reaction (flushing) disappeared and after 2-3 minutes the patient was symptom free (event stop time reported as [redacted] (b) (6) at 11:55). After the patient regained consciousness, blood pressure was 80/50 mmHg. The patient reported that he had drunk and eaten too little.

After administration of fluids (Sterofundin), patisiran (ALN-TTR02) infusion was restarted at a lower rate (60-120 ml/hour) and was tolerated well by the patient. No disturbance of electrolytes or hypoglycaemia was found. The patient also had hypertension (blood pressure 200/130 mmHg) due to the patient’s wheelchair being stolen on the same day (reported as a non-serious adverse event, MCN 2018DEALNTTR020300), the patient was given amlodipine as treatment for this event. The patient was discharged from hospital 2 hours after patisiran infusion was stopped upon request of the patient. The physician reported the collapse was a result of hypotension due to infusion related reaction combined with dehydration, low ejection fraction (EF) and polyneuropathy (PNP). The physician noted that orthostatic, rhythmogenic and vasovagal syncope were clinically unlikely” (MedWatch Report # 2018DEALNTTR020299).

Reviewer Comment: The IRRs observed following patisiran treatment, including the reduced premedication regimen, were generally tolerable and did not lead to treatment cessation. Although no anaphylactic reactions were observed in the patisiran development program, there remains a potential risk for anaphylaxis, especially in the setting of inadequate premedication. As seen above, SAEs such as syncope can occur despite the premedication regimen. These risks should be clearly stated in the patisiran label.

8.5.3. Extravasation

Extravasation was observed in <0.5% of infusions in clinical studies. Infusion site extravasation led to infusion interruption in 2% of patients. Signs and symptoms included phlebitis or thrombophlebitis, infusion or injection site swelling, dermatitis (subcutaneous inflammation), cellulitis, erythema or injection site redness, burning sensation, or injection site pain. There were two reports of SAEs in Study 004 associated with extravasation of patisiran (post-infusion cellulitis/superficial thrombophlebitis, and dermatitis).

Reviewer Comment: The risk of extravasation should be described in the patisiran label.

8.5.4. Metabolic Bone Disorders

An analysis of events mapping to the Osteoporosis/osteopenia SMQ was performed by the applicant because patients with hATTR amyloidosis have been noted to have decreased bone mineral density. The results for Study 004 are shown in the following table, copied from the submission. *The small difference between placebo and patisiran groups is of unclear clinical significance.*

Table 84: Adverse Events in the Osteoporosis/Osteopenia SMQ (ALN-TTR02-004 Safety Population). Source: Summary of Clinical Safety, p. 97

High Level Term/ Preferred Term	Number of Patients (%)[a]/Events[b]	
	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
At Least 1 AE	9 (11.7)/11	14 (9.5)/14
Injury, poisoning and procedural complications	3 (3.9)/3	3 (2.0)/3
Cervical vertebral fracture	0	1 (0.7)/1
Spinal compression fracture	2 (2.6)/2	1 (0.7)/1
Wrist fracture	1 (1.3)/1	1 (0.7)/1
Musculoskeletal and connective tissue disorders	7 (9.1)/8	11 (7.4)/11
Osteopenia	1 (1.3)/1	4 (2.7)/4
Osteoporosis	7 (9.1)/7	7 (4.7)/7

Abbreviations: AE= adverse event; MedDRA=Medical Dictionary for Regulatory Activities; PT=preferred term; SOC=system organ class; SMQ=standardized MedDRA query.
Note: The Medical Dictionary for Regulatory Activities (MedDRA) version 18.0 is used to code AEs. All AEs are included from the Osteoporosis/Osteopenia – Comprehensive Search SMQ.
[a.] If a patient experienced more than 1 event in a given SOC, that patient is counted once for the SOC. If a patient experienced more than 1 event with a given PT, that patient is counted only once for that PT. Percentages are based out of the total number of subjects (N) who were on study at the start of the indicated exposure duration category.
[b.] The total number of events for all patients; a patient can be counted more than once if the patient has multiple events.

8.5.5. Ocular Events

Patients were administered ophthalmology exams throughout the patisiran studies and an evaluation of ocular disorder AEs was conducted because TTR reduction in patients treated with patisiran-LNP is associated with concomitant reduction in circulating serum levels of Retinol binding protein and vitamin A. Abnormal visual adaptation to darkness (night blindness) is a symptom of vitamin A deficiency.

Ophthalmology was consulted for this application to evaluate the clinical study adverse event data and electroretinogram (ERG) data for evidence of ocular toxicity related to vitamin A deficiency. The reader is referred to that consult review for a full analysis and discussion. The consult concluded that there were no ocular vitamin A related abnormalities identified in the clinical trials.

Table 85: Eye Disorder Treatment-Emergent Adverse Events in Study 004. Source: Study 004 CSR

System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Eye disorders	20 (26.0)/26	41 (27.7)/61
Blepharitis	0	1 (0.7)/1
Cataract	5 (6.5)/5	8 (5.4)/10
Cataract nuclear	0	2 (1.4)/3
Cataract subcapsular	0	1 (0.7)/1
Conjunctival haemorrhage	3 (3.9)/3	1 (0.7)/1
Conjunctivitis allergic	0	1 (0.7)/1
Corneal erosion	0	1 (0.7)/1
Corneal opacity	0	1 (0.7)/1
Diplopia	1 (1.3)/1	1 (0.7)/1
Dry eye	2 (2.6)/2	7 (4.7)/7
Eye irritation	0	2 (1.4)/2
Eye swelling	0	1 (0.7)/1
Eyelid oedema	1 (1.3)/1	0
Eyelid ptosis	1 (1.3)/1	0
Glaucoma	1 (1.3)/1	2 (1.4)/3
Intraocular haematoma	0	1 (0.7)/1
Keratitis	0	2 (1.4)/2
Macular scar	0	1 (0.7)/1
Maculopathy	0	1 (0.7)/2
Night blindness	1 (1.3)/1	0
Ocular discomfort	0	1 (0.7)/2
Ocular hyperaemia	1 (1.3)/1	0
Optic atrophy	0	1 (0.7)/1
Optic nerve cupping	1 (1.3)/1	1 (0.7)/1
Pinguecula	0	1 (0.7)/1
Retinal degeneration	1 (1.3)/1	0
Retinal detachment	0	1 (0.7)/1
Retinal disorder	1 (1.3)/1	0
Retinal pigment epitheliopathy	0	1 (0.7)/1
Retinal scar	0	1 (0.7)/1
Trichiasis	1 (1.3)/1	0
Ulcerative keratitis	1 (1.3)/1	0
Vision blurred	1 (1.3)/1	4 (2.7)/4
Visual acuity reduced	2 (2.6)/2	1 (0.7)/1
Visual impairment	0	2 (1.4)/2
Vitreous floaters	1 (1.3)/1	3 (2.0)/3
Vitreous haemorrhage	0	1 (0.7)/1
Vitreous opacities	1 (1.3)/1	2 (1.4)/2
Vitritis	0	1 (0.7)/1

8.5.6. **Depression/Suicidality**

The applicant assessed depression and suicidality through a standardized MedDRA query and through the Columbia-Suicide Severity Rating Scale (C-SSRS) questionnaire because hATTR amyloidosis patients may experience depression related to the disease's high morbidity and mortality. In addition, the prospective assessment of suicidality is standard FDA policy for clinical trials of central nervous system drugs. The following tables, copied from the submission, summarize the results for the placebo-controlled Study 004. *These results show that there is no signal of concern for depression and suicidality in the patisiran group.*

Table 86: Incidence of Treatment-Emergent Adverse Events using Depression and Suicide/Self-Injury SMQ by Preferred Term (Safety Population). Source: Study 004 CSR, p. 1062

Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
At Least 1 AE	8 (10.4)/21	8 (5.4)/10
Depression	6 (7.8)/8	5 (3.4)/5
Disturbance in attention	0	1 (0.7)/3
Initial insomnia	0	1 (0.7)/1
Memory impairment	0	1 (0.7)/1
Poor quality sleep	1 (1.3)/1	0
Psychomotor hyperactivity	1 (1.3)/12	0

Table 87: Shift from Baseline in C-SSRS Categories (Safety Population). Source: Study 004 CSR, p. 258

Treatment Group	Baseline Category	Worst Post-Baseline N (%)				
		No Suicidal Ideation or Behavior	Suicidal Ideation	Suicidal Behavior	Missing	Total
Patisiran-LNP 0.3 mg/kg	No suicidal ideation or behavior	95 (64.2)	9 (6.1)	0	8 (5.4)	112 (75.7)
	Suicidal ideation	8 (5.4)	11 (7.4)	0	2 (1.4)	21 (14.2)
	Suicidal behavior	0	0	0	0	0
	Missing	6 (4.1)	2 (1.4)	0	7 (4.7)	15 (10.1)
	Total	109 (73.6)	22 (14.9)	0	17 (11.5)	148 (100.0)
Placebo	No suicidal ideation or behavior	43 (55.8)	4 (5.2)	0	5 (6.5)	52 (67.5)
	Suicidal ideation	3 (3.9)	10 (13.0)	1 (1.3)	0	14 (18.2)
	Suicidal behavior	1 (1.3)	0	1 (1.3)	0	2 (2.6)
	Missing	3 (3.9)	5 (6.5)	0	1 (1.3)	9 (11.7)
	Total	50 (64.9)	19 (24.7)	2 (2.6)	6 (7.8)	77 (100.0)

Abbreviations: C-SSRS=Columbia-Suicide Severity Rating Scale Questionnaire

8.5.7. Cardiac Deaths

Although there was a higher percentage of total deaths in the placebo group of Study 004, the following table shows that there was a higher percentage of cardiac deaths in the patisiran group.

Table 88: Study 004 Results: More Deaths in Placebo, but More Cardiac Deaths in Drug Group

	APOLLO Placebo (N=77)	APOLLO Patisiran 0.3 mg/kg (N=148)
Deaths, n (%)	6 (7.8)	7 (4.7)
Percent difference (95% CI) (patisiran – placebo)		-3.1 (-11.0, 3.3)
Odds ratio (95% CI) (patisiran/placebo)		0.59 (0.19, 1.89)

	APOLLO Placebo (N=77)	APOLLO Patisiran 0.3 mg/kg (N=148)
Cardiac Deaths, n (%)	1 (1.3)	7 (4.7)
Percent difference (95% CI) (patisiran – placebo)		3.4 (-1.6, 8.0)
Odds ratio (95% CI) (patisiran/placebo)		3.77 (0.65, 71.20)

The applicant had these cases re-adjudicated by an independent blinded committee to determine if the causes of death with “cardiovascular” or “non-cardiovascular.” The results of that re-adjudication suggested that 2 additional placebo deaths could be considered as cardiovascular in nature. However, these deaths were notably caused by strokes and not CHF or arrhythmias. All of the patisiran deaths were related to CHF or arrhythmias. Therefore, the deaths due to cardiac causes such as CHF or arrhythmia remain at 7 (4.7%) to 1 (1.3%) in the drug versus placebo comparison.

The Val30Met genetic mutation is most commonly associated with hATTR polyneuropathy. Note that patisiran group deaths were all in patients with non-Val30Met genotypes, including

two patients with the Thr60Ala mutation which has been associated with higher mortality risk (Swiecicki et al., 2015).

Reviewer comment: Death from ATTR amyloidosis occurs most often because of cardiac dysfunction. Cardiac deaths occurred more often in the patisiran group, but these deaths included patients with gene mutations associated with a higher mortality. The small numbers of cardiac deaths, combined with the lower overall incidence of death on treatment, make this finding difficult to interpret.

8.6. Safety Analyses by Demographic Subgroups

8.6.1. Age: Adverse Events Analysis

All Phase 2 and Phase 3 patisiran studies were conducted with patients with hATTR amyloidosis over the age of 18.

In Study 004 and in the pooled studies, patients ranged in age from 24 to 83 years at the time of enrollment. As seen in the following tables copied from the submission, there *were no clinically significant differences in the proportions of patients with AEs or with SAEs between the patisiran-LNP and placebo groups as a function of age subgroup for Study 004.*

Table 89: Incidence of Treatment-Emergent Adverse Events by Age Subgroup. Source: Study 004 CSR, p. 1106

		Number of patients (%)[1]/Events[2]	
Subgroup (Level)	System Organ Class/ Preferred Term	Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Age (<65)	Number of Patients	44	86
	At Least 1 AE[3]	43 (97.7)/681	83 (96.5)/1106
Age (>=65)	Number of Patients	33	62
	At Least 1 AE[3]	32 (97.0)/550	60 (96.8)/972

Table 90: Incidence of Serious Treatment-Emergent Adverse Events by Age Subgroup. Source: Study 004 CSR, p. 1391

Subgroup (Level)	System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Age (<65)	Number of Patients	44	86
	At Least 1 AE[3]	14 (31.8)/55	24 (27.9)/36
Age (>=65)	Number of Patients	33	62
	At Least 1 AE[3]	17 (51.5)/44	30 (48.4)/65

8.6.2. Sex: Adverse Events Analysis

In the overall pooled experience (218 patients), 160 (73.4%) patients were male and 58 (26.6%) patients were female. The proportion of male patients and female patients experiencing AEs was 93.8% and 98.3%, respectively. The proportion of male patients and female patients experiencing SAEs was 40.6% and 39.7%, respectively.

In Study 004, 167 (74.2%) patients were male, including 109 of 148 patients (73.6%) in the patisiran group and 58 of 77 patients (75.3%) in the placebo group.

As seen in the following tables copied from the submission, there *were no clinically significant differences in the proportions of patients with AEs or with SAEs between the patisiran-LNP and placebo groups as a function of sex subgroup for Study 004.*

Table 91: Incidence of Treatment-Emergent Adverse Events by Sex Subgroup. Source: Study 004 CSR, p. 1170

Subgroup (Level)	System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Sex (M)	Number of Patients	58	109
	At Least 1 AE[3]	56 (96.6)/895	107 (98.2)/1509
Sex (F)	Number of Patients	19	39
	At Least 1 AE[3]	19 (100.0)/336	36 (92.3)/569

Table 92: Incidence of Serious Treatment-Emergent Adverse Events by Sex Subgroup. Source: Study 004 CSR, p. 1413

Subgroup (Level)	System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Sex (M)	Number of Patients	58	109
	At Least 1 AE[3]	24 (41.4)/63	41 (37.6)/79
Sex (F)	Number of Patients	19	39
	At Least 1 AE[3]	7 (36.8)/36	13 (33.3)/22

8.6.3. Race: Adverse Events Analysis

In the overall pooled experience, 168 (77.1%) of 218 patients were White/Caucasian. The proportion of AEs in White/Caucasian and non-White patients was 95.8% (161 of 168 patients) and 91.5% (43 of 47 patients), respectively. The proportion of White/Caucasian and non-White patients experiencing SAEs was 41.7% and 34.0%, respectively.

In Study 004, 163 (72.4%) patients were White/Caucasian, including 113 of 148 patients (76.4%) in the patisiran-LNP group and 50 of 77 patients (64.9%) in the placebo group. As seen in the following tables copied from the submission, *there were no clinically significant differences in the proportions of patients with AEs or with SAEs between the patisiran-LNP and placebo groups as a function of race subgroup for Study 004.*

Table 93: Incidence of Treatment-Emergent Adverse Events by Race Subgroup. Source: Study 004 CSR, p. 1155

Subgroup (Level)	System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Race (White)	Number of Patients	50	113
	At Least 1 AE[3]	49 (98.0)/832	110 (97.3)/1670
Race (Non-White)	Number of Patients	26	33
	At Least 1 AE[3]	25 (96.2)/388	31 (93.9)/379

Table 94: Incidence of Serious Treatment-Emergent Adverse Events by Race Subgroup. Source: Study 004 CSR, p. 1402

Subgroup (Level)	System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Race (White)	Number of Patients	50	113
	At Least 1 AE[3]	20 (40.0)/57	43 (38.1)/83
Race (Non-White)	Number of Patients	26	33
	At Least 1 AE[3]	10 (38.5)/41	10 (30.3)/17

8.6.4. Weight: Adverse Events Analysis

In the overall pooled experience, 112 (51.4%) patients out of the 218 patients weighed ≥65 kg. The proportion of patients <65 kg and patients ≥65 kg experiencing AEs was 94.3% (100 of 106 patients) and 95.5% (107 of 112 patients), respectively. The proportion of patients <65 kg and patients ≥65 kg experiencing SAEs was 39.6% and 41.1%, respectively.

In Study 004, 114 (50.7%) patients were ≥65 kg, including 75 of 148 patients (50.7%) in the patisiran-LNP group and 39 of 77 patients (50.6%) in the placebo group.

As seen in the following tables copied from the submission, *there were no clinically significant differences in the proportions of patients with AEs or with SAEs between the patisiran-LNP and placebo groups as a function of weight subgroup for Study 004.*

Table 95: Incidence of Treatment-Emergent Adverse Events by Weight Subgroup. Source: Study 004 CSR, p. 1349

Subgroup (Level)	System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Weight (<65 kg)	Number of Patients	38	73
	At Least 1 AE[3]	37 (97.4)/661	70 (95.9)/1014
Weight (>=65 kg)	Number of Patients	39	75
	At Least 1 AE[3]	38 (97.4)/570	73 (97.3)/1064

Table 96: Incidence of Serious Treatment-Emergent Adverse Events by Weight Subgroup. Source: Study 004 CSR, p. 1460

Subgroup (Level)	System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Weight (<65 kg)	Number of Patients	38	73
	At Least 1 AE[3]	14 (36.8)/62	27 (37.0)/51
Weight (>=65 kg)	Number of Patients	39	75
	At Least 1 AE[3]	17 (43.6)/37	27 (36.0)/50

8.6.5. Genotype: Adverse Events Analysis

In the overall pooled experience, 97 (44.5%) of the 218 patients had the V30M mutation. The proportion of V30M patients and non-V30M patients experiencing AEs was 96.9% (94 of 97 patients) and 93.4% (113 of 121 patients), respectively. The proportion of V30M patients and non-V30M patients experiencing SAEs was 41.2% and 39.7%, respectively.

In Study 004, 96 (42.7%) patients had the V30M mutation, including 56 of 148 patients (37.8%) in the patisiran-LNP group and 40 of 77 patients (51.9%) in the placebo group.

As seen in the following tables copied from the submission, *there were no clinically significant differences in the proportions of patients with AEs or with SAEs between the patisiran-LNP and placebo groups as a function of genotype subgroup for Study 004.*

Table 97: Incidence of Treatment-Emergent Adverse Events by Genotype Subgroup. Source: Study 004 CSR, p. 1211

Subgroup (Level)	System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Genotype (V30M)	Number of Patients	40	56
	At Least 1 AE[3]	40 (100.0)/640	55 (98.2)/727
Genotype (Non-V30M)	Number of Patients	37	92
	At Least 1 AE[3]	35 (94.6)/591	88 (95.7)/1351

Table 98: Incidence of Serious Treatment-Emergent Adverse Events by Genotype Subgroup. Source: Study 004 CSR, p. 1424

Subgroup (Level)	System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Genotype (V30M)	Number of Patients	40	56
	At Least 1 AE[3]	17 (42.5)/42	22 (39.3)/36
Genotype (Non-V30M)	Number of Patients	37	92
	At Least 1 AE[3]	14 (37.8)/57	32 (34.8)/65

8.6.6. FAP Stage: Adverse Events Analysis

The Familial Amyloidotic Polyneuropathy (FAP) Stage is defined in the following table, copied from the submission.

Table 99: Familial Amyloidotic Polyneuropathy Stage Descriptions. Source: Clinical Overview, p. 35

Stage	Description
0	No symptoms
I	Unimpaired ambulation; mostly mild sensory, motor, and autonomic neuropathy in the lower limbs
II	Assistance with ambulation required, mostly moderate impairment progression to the lower limbs, upper limbs, and trunk
III	Wheelchair-bound or bedridden; severe sensory, motor, and autonomic involvement of all limbs

In the overall pooled experience (218 patients), 103 (47.2%) patients were FAP Stage I at baseline and 115 (52.8%) patients were FAP Stage II/III. The proportion of FAP Stage I patients and Stage II/III patients experiencing AEs was 95.1% (98 of 103 patients) and 94.8% (109 of 115 patients), respectively. The frequency proportion of patients with SAEs in FAP Stage I patients and Stage II/III patients was 30.1% and 49.6%, respectively.

In Study 004, 104 (46.2%) patients were FAP Stage I at baseline, including 67 of 148 patients (45.3%) in the patisiran-LNP group and 37 of 77 patients (48.1%) in the placebo group. The remainder of patients was in FAP Stage II/III.

As seen in the following tables copied from the submission, *there were no clinically significant differences in the proportions of patients with AEs or with SAEs between the patisiran-LNP and placebo groups as a function of FAP Stage subgroup for Study 004.*

Table 100: Incidence of Treatment-Emergent Adverse Events by FAP Stage Subgroup. Source: Study 004 CSR, p. 1305

		Number of patients (%)[1]/Events[2]	
Subgroup (Level)	System Organ Class/ Preferred Term	Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
FAP Stage (I)	Number of Patients	37	67
	At Least 1 AE[3]	35 (94.6)/616	66 (98.5)/962
FAP Stage (II/III)	Number of Patients	40	81
	At Least 1 AE[3]	40 (100.0)/615	77 (95.1)/1116

Table 101: Incidence of Severe Treatment-Emergent Adverse Events by FAP Stage Subgroup. Source: Study 004 CSR, p. 1448

		Number of patients (%)[1]/Events[2]	
Subgroup (Level)	System Organ Class/ Preferred Term	Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
FAP Stage (I)	Number of Patients	37	67
	At Least 1 AE[3]	10 (27.0)/37	18 (26.9)/31
FAP Stage (II/III)	Number of Patients	40	81
	At Least 1 AE[3]	21 (52.5)/62	36 (44.4)/70

8.6.7. Cardiac Subpopulation: Adverse Events Analysis

In order to assess the safety of patisiran in hATTR patients with cardiac disease, the applicant conducted safety analyses in predefined subsets of patients in Studies 003 and 004 with pre-existing cardiac disease due to amyloidosis, defined as follows.

In Study 003, 11 patients were included in the cardiac subpopulation, defined as having left ventricular wall thickness (LVWT) ≥1.3 cm, no aortic valve disease, and normotensive or hypertension that is well-controlled, based on investigator determination at the time of screening. Four patients (14.8%) had AEs in the cardiac disorders system organ class, with two SAEs (myocardial infarction and atrioventricular block).

In Study 004, 90 of 148 (~61%) patisiran and 36 of 77 (~47%) placebo patients were identified for the cardiac subpopulation, defined as having a baseline LVWT ≥1.3 cm and no aortic valve disease or hypertension in their medical history. The results of the cardiac subpopulation safety analysis for the placebo-controlled Study 004 are shown in the following table, copied from the submission.

Table 102: Summary of Cardiac Safety (ALN-TTR02-004 Cardiac Subpopulation[a]). Source: Summary of Clinical Safety, p. 128.

	Number of Patients (%)[a]/Events[b]	
Type of Adverse Event	Placebo (N=36)	Patisiran-LNP (N=90)
Any AE in the Cardiac disorders SOC	13 (36.1)/26	29 (32.2)/42
Any SAE in the Cardiac disorders SOC	4 (11.1)/7	13 (14.4)/16
Any AE in the Cardiac arrhythmias (HGLT)	11 (30.6)/18	17 (18.9)/24
Cardiac conduction disorders HLT	3 (8.3)/3	6 (6.7)/8
Rate and rhythm disorders NEC HLT	0	2 (2.2)/2
Supraventricular arrhythmias HLT	9 (25.0)/12	11 (12.2)/11
Ventricular arrhythmias and cardiac arrest HLT	3 (8.3)/3	3 (3.3)/3
Any AE in the Torsades de pointes SMQ[c]	5 (13.9)/5	7 (7.8)/7
Any AE in the Cardiac failure SMQ (narrow)	2 (5.6)/5	10 (11.1)/12
Any AE in the Cardiac failure SMQ (broad)	10 (27.8)/27	33 (36.7)/69

Abbreviations: AE=adverse event; HGLT=high level group term; HLT=high level term; NEC HLT=not elsewhere classified high level term; SMQ=standardized MedDRA queries; SOC=system organ class
Note: Cardiac subpopulation: patients with pre-existing cardiac amyloid involvement (ie, patients with baseline left ventricular [LV] wall thickness ≥ 1.3 cm and no aortic valve disease or hypertension in medical history)
[a] If a patient experienced more than 1 event in a given SOC, HGLT, HLT, or SMQ, that patient is counted once for the SOC, HGLT, HLT, or SMQ. If a patient experienced more than 1 event with a given PT, that patient is counted only once for that preferred term. Percentages are based out of the total number of subjects (N) who were on study at the start of the indicated exposure duration category.
[b] The total number of events for all patients; a patient can be counted more than once if the patient has multiple events.
[c] Torsades de Pointes SMQ is a search for reported events that may be associated with Torsades. It does not mean that these are confirmed events of Torsades de pointes; no cases of Torsades de pointes have been reported.

The following tables, generated from the submitted data, show the cardiac adverse events and serious adverse events in the total population for Study 004.

Table 103: Cardiac Adverse Events in Total Population for Study 004. Source: FDA analysis

		Patisiran 0.3 mg/kg		PLACEBO	
		N=148		N=77	
AEBODSYS	AEDECOD	N Rows	Percent	N Rows	Percent
Cardiac disorders	Atrial fibrillation	13	8.8%	5	6.5%
	Cardiac amyloidosis	4	2.7%	1	1.3%
	Cardiac failure congestive	5	3.4%	2	2.6%
	Atrioventricular block complete	3	2.0%	.	.
	Bradycardia	3	2.0%	.	.

Table 104: Cardiac Serious Adverse Events in Total Population for Study 004. Source: FDA analysis

		TRT01A			
		Patisiran 0.3 mg/kg		PLACEBO	
		N=148		N=77	
AEBODSYS	AEDECOD	N Rows	Percent	N Rows	Percent
Cardiac disorders	Atrial fibrillation	2	1.35135%	1	1.29870%
	Cardiac amyloidosis	2	1.35135%	1	1.29870%
	Cardiac arrest	2	1.35135%	1	1.29870%
	Cardiac failure	3	2.02703%	2	2.59740%
	Cardiac failure congestive	3	2.02703%	2	2.59740%
	Conduction disorder	1	0.67568%	1	1.29870%
	Arteriosclerosis coronary artery	1	0.67568%	.	.
	Atrioventricular block	1	0.67568%	.	.
	Atrioventricular block complete	3	2.02703%	.	.
	Cardiogenic shock	1	0.67568%	.	.
	Cardiomyopathy	1	0.67568%	.	.
	Pulseless electrical activity	1	0.67568%	.	.
	Ventricular dyssynchrony	1	0.67568%	.	.
	Ventricular fibrillation	1	0.67568%	.	.

There was a greater percentage of patients with AEs of conduction disorders (atrial fibrillation, atrioventricular block complete) and congestive cardiac failure in the patisiran group As previous described, the imbalance in the SAEs for AV heart block should be described in product labeling. For a discussion of cardiac deaths, see section 8.4.1.

8.6.8. Geographic Region: Adverse Events Analysis

Adverse events were compared among three geographic regions: North America (United States and Canada); Western Europe (Germany, Spain, France, United Kingdom, Italy, Netherlands, Portugal, Sweden); and Rest of World (ROW: Bulgaria, Cyprus, Turkey, Japan, Korea, Taiwan, Mexico, Argentina, and Brazil).

As seen in the following table based on the applicant's data, *there does not appear to be a meaningful difference among the groups, except for more SAEs in the placebo group in North America and in the patisiran group in Western Europe. The clinical significance of these differences is unclear, but may reflect differences in regional medical practice.*

Table 105: Adverse Events and Serious Adverse Events by Geographic Region. Source: Summary of Clinical Safety, p. 130.

Region	**Adverse Events (%)**		**Serious Adverse Events (%)**	
	Placebo	Patisiran	Placebo	Patisiran
North America	100.0	97.3	70.0	29.7
Western Europe	97.2	98.4	38.9	46.8
Rest of World	96.8	93.9	32.3	28.6

8.7. Specific Safety Studies/Clinical Trials

No specific clinical safety studies were performed in the patisiran development program.

8.8. Additional Safety Explorations

8.8.1. Human Carcinogenicity or Tumor Development

In Study 004, a total of 7 patients reported malignancies, 3 (2.0%) in the patisiran-LNP group and 4 (5.2%) in the placebo group, as shown in the following table copied from the submission.

Table 106: Malignancies by Preferred Term (ALN-TTR02-004 Safety Population). Source: Summary of Clinical Safety, p. 89

High Level Term/ Preferred Term	Number of Patients (%)[a]/Events[b] Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
At Least 1 AE	4 (5.2)/5	3 (2.0)/6
Bladder neoplasms malignant	0	1 (0.7)/2
Bladder cancer	0	1 (0.7)/2
Colorectal neoplasms malignant	2 (2.6)/2	0
Colon cancer metastatic	1 (1.3)/1	0
Colorectal cancer metastatic	1 (1.3)/1	0
Prostatic neoplasms malignant	1 (1.3)/1	0
Prostate cancer	1 (1.3)/1	0
Skin melanomas (excluding ocular)	1 (1.3)/1	0
Malignant melanoma in situ	1 (1.3)/1	0
Skin neoplasms malignant and unspecified (excluding melanoma)	1 (1.3)/1	2 (1.4)/4
Atypical fibroxanthoma	0	1 (0.7)/2
Basal cell carcinoma	1 (1.3)/1	1 (0.7)/2

Abbreviations: AE=adverse event.
Note: The Medical Dictionary for Regulatory Activities (MedDRA) version 18.0 is used to code adverse events. All AEs are included from the Malignant or Unspecified Tumors standardized MedDRA query (SMQ).
[a] If a patient experienced more than 1 event in a given system organ class (SOC), that patient is counted once for the SOC. If a patient experienced more than 1 event with a given preferred term (PT), that patient is counted only once for that PT. Percentages are based out of the total number of subjects (N) who were on study at the start of the indicated exposure duration category.
[b] The total number of events for all patients; a patient can be counted more than once if the patient has multiple events.

Of the 3 patients in the patisiran group with malignancies, 2 patients had recurrences of previous skin neoplasms. The third patient had a newly diagnosed case of bladder cancer that resolved on treatment. *There was a higher percentage of malignancies in the placebo group. There does not appear to be any increased risk of cancer associated with patisiran treatment.*

8.8.2. Human Reproduction and Pregnancy

The applicant has not conducted any studies of patisiran-LNP in pregnant or lactating women. Pregnant or lactating women were excluded from participation in all clinical studies. As of the data cutoff date for Study 006 (December 1, 2017), there have been no reported pregnancies in the patisiran-LNP clinical development program.

8.8.3. Pediatrics and Assessment of Effects on Growth

No data are available for pediatric patients (<18 years of age). Symptom onset for hATTR amyloidosis occurs between 20 and 70 years of age.

8.8.4. Overdose, Drug Abuse Potential, Withdrawal, and Rebound

There were two protocol deviations in Study 004 in which patients received an incorrect dosage of study drug with a <10% increase over the correct dosage. There were no AEs or SAEs associated with the event and both patients continued in the study.

The Agency told the applicant in the Type B pre-NDA meeting on November 13, 2017, that "there is no need to include an assessment of the abuse potential of the drug or a proposal for scheduling the drug in your NDA. The drug does not affect the CNS, it is not chemically or pharmacologically similar to other drugs with known abuse potential, and it does not produce psychoactive effects such as sedation, euphoria, and mood changes."

8.9. Safety in the Postmarket Setting

8.9.1. Safety Concerns Identified Through Postmarket Experience

Patisiran is not approved or marketed in any country.

8.10. Integrated Assessment of Safety

The overall conclusion of this safety review is that the safety profile of patisiran is acceptable given the evidence of efficacy in the treatment of hATTR-PN and the poor prognosis for hATTR amyloidosis patients. The most commonly observed (≥ 10%) adverse events associated with the use of patisiran in the 18-month placebo-controlled study were upper respiratory tract infections and infusion-related reactions. Patisiran reduces vitamin A levels in the body. All patients received the recommended daily amount of vitamin A as a supplement and no ocular AEs related to low vitamin A levels were observed. Although there were more respiratory infections (URI, Cold, Rhinitis, Upper respiratory tract infection, Flu-Like illness) in the patisiran group (4%) compared to placebo (0%), it is unclear if this difference was related to low vitamin A levels.

Infusion related reactions included flushing (6.0% across all studies), back pain (5.5%), nausea (3.7%), dyspnea (2.8%), and headache (2.3%). Hypotension occurred in 1.4% of patisiran patients in the placebo-controlled study, compared to 0% in the placebo group. One patient in the expanded access program experienced hypotension and syncope during the patisiran infusion. In order to decrease the risk of infusion related reactions, all clinical study subjects received premedication regimens that included corticosteroids, H1/H2 blockers, and

paracetamol or equivalents. Two patients had severe adverse events that were most likely related to corticosteroid use: osteomyelitis, osteopenia, and tibia fracture. Additional adverse events that were possibly due to premedication included dizziness, insomnia, and somnolence. There were two reports of SAEs in Study 004 associated with extravasation of patisiran (post-infusion cellulitis/superficial thrombophlebitis, and dermatitis).

Consistent with the natural history of hATTR amyloidosis, the adverse event that caused the most patients to stop taking patisiran across all clinical studies was cardiac failure (2 patients in the placebo-controlled study, 1.4%). Review of cardiac adverse events generally found no clinically significant difference in cardiac AEs and SAEs between the placebo and patisiran cardiac subpopulations or total study populations. However, 4 SAEs of AV heart block, including 3 cases of complete AV block, occurred patisiran treated subjects in Study 004 compared to no cases in placebo. In the 18-month placebo-controlled study, mortality was numerically lower in the patisiran group (4.7%) than in the placebo group (7.8%) (deaths from cardiac causes such as CHF or arrhythmia occurred in 6/148 patisiran-treated subjects compared to 1/77 placebo-treated subjects). However, the number of deaths were too small to make any clear interpretation of these results.

This reviewer concludes that the safety profile of patisiran is acceptable, but that the label should include under Warnings and Precautions a description of the risks of infusion-related reactions and the need for vitamin A supplementation to avoid possible vitamin A deficiency. Dosage and Administration in labeling should include the premedication regimen used in the placebo-controlled study of patisiran to reduce the risk of infusion-related reactions.

9. Advisory Committee Meeting and Other External Consultations

This section is not applicable to this review.

10. Labeling Recommendations

10.1. Prescription Drug Labeling

As discussed in Section 4.5, the Office of Clinical Pharmacology recommends a fixed-dose of

patisiran 0.3 mg/kg IV infusion over 80 min every 3 weeks. For patients weighing ≥100 kg, the dose is capped to 30 mg.

Note that patisiran is not administered by patients themselves or their family members, but must be given intravenously by a supervising healthcare professional in a setting with available resuscitation equipment due to the risk of infusion-related reactions.

The SAEs of AV block will be described in the Adverse Reactions section.

The ophthalmology consult recommends, based primarily on Study 004, that the following ocular adverse reactions, which may be related to the use of patisiran, should be included in the labeling: dry eye, blurred vision and vitreous floaters.

This reviewer recommends describing the following issues in the Warnings and Precautions section of labeling: infusion-related reactions, extravasation, and reduced serum vitamin A levels with recommended supplementation.

Dosage and Administration should include a description of the premedication regimen used in the placebo-controlled Study 004.

10.2. Nonprescription Drug Labeling

Not applicable.

11. Risk Evaluation and Mitigation Strategies (REMS)

This section is not applicable to this review.

12. Postmarketing Requirements and Commitments

As discussed in a teleconference between Alnylam and members of the OPQ review team, in the event of an approval action, a postmarketing commitment for development of an alternative in vitro release method for patisiran will be requested.

If an approval action is taken, the Agency is considering an FDAAA postmarketing requirement to evaluate pregnancy outcomes.

13. Appendices

13.1. References

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Diabetes Technol Ther, 7(3): 497-508.

Vinik, E., et al. (2014)
Norfolk QOL-DN: validation of a patient reported outcome measure in transthyretin familial amyloid polyneuropathy.
J Peripher Nerv Syst, 19(2): 104-114.

13.2. Financial Disclosure

Covered Clinical Study: ALN-TTR02-002

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 63		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 4		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0 Significant payments of other sorts: 4 Proprietary interest in the product tested held by investigator: 0 Significant equity interest held by investigator in Study: 0 Sponsor of covered study: 0		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): 10		
Is an attachment provided with the	Yes ☒	No ☐ (Request explanation

reason:		from Applicant)

Covered Clinical Study: ALN-TTR02-003

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 61		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 4		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0 Significant payments of other sorts: 4 Proprietary interest in the product tested held by investigator: 0 Significant equity interest held by investigator in Study: 0 Sponsor of covered study: 0		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): 9		
Is an attachment provided with the reason:	Yes ☒	No ☐ (Request explanation from Applicant)

Covered Clinical Study: ALN-TTR02-004

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from

		Applicant)
Total number of investigators identified: 370		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 6		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0 Significant payments of other sorts: 6 Proprietary interest in the product tested held by investigator: 0 Significant equity interest held by investigator in Study: 0 Sponsor of covered study: 0		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): 51		
Is an attachment provided with the reason:	Yes ☒	No ☐ (Request explanation from Applicant)

Covered Clinical Study: ALN-TTR02-006

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 292		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455):		

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Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: <u>0</u>

Significant payments of other sorts: <u>6</u>

Proprietary interest in the product tested held by investigator: <u>0</u>

Significant equity interest held by investigator in Study: 0

Sponsor of covered study: <u>0</u></td></tr>
<tr><td>Is an attachment provided with details of the disclosable financial interests/arrangements:</td><td>Yes ☒</td><td>No ☐ (Request details from Applicant)</td></tr>
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<tr><td>Is an attachment provided with the reason:</td><td>Yes ☒</td><td>No ☐ (Request explanation from Applicant)</td></tr>
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13.3. Summary Table of All Deaths

Table 107: Summary of All Deaths in Patisiran-LNP Clinical Trials. Source: Summary of Clinical Safety, p. 56

Patient Number Age/Gender Genotype	Cardiac Sub-group	Serious Adverse Event reported as Fatal	Adjudication (Subgroup)[a]	Study day(s) to Last dose of Study Drug	Study day (s) to Date of Death	Relevant Current and Historical Medical Condition
Study 004: Placebo-Controlled Study						
Patisiran-LNP (n=7; 4.7%)						
[redacted] (b) (6) 66/M Ala97Ser	Yes	Cardiac arrest, Cardiac failure congestive	CV (heart failure)	191	194	004 Baseline: FAP stage I, PND score II; NYHA class II, NT-proBNP 647.35 pmol/L, MLVWT 2.32cm Med Hx: congestive cardiac failure, cardiac amyloidosis, diabetes mellitus, atrial flutter, atrioventricular block Worsening CHF. Autopsy: Death due to complications of systemic TTR amyloidosis with extensive cardiac involvement.
[redacted] (b) (6) 66/M Thr60Ala	Yes	Sudden cardiac death	CV (presumed sudden death)	64	169[b]	004 Baseline: FAP stage II, PND score IIIA; NYHA class I, NT-proBNP 876.15 pmol/L, MLVWT 2.08cm Med Hx: congestive cardiac failure, atrial fibrillation, atrial flutter, cardiac ablation, first degree atrioventricular block, cardiac amyloidosis, chronic kidney disease Patient with course complicated by heel ulcer, osteomyelitis, vancomycin toxicity resulting in acute kidney injury, CHF exacerbation, UTI, CVA, acute heart failure.
[redacted] (b) (6) 67/M Thr60Ala	Yes	Sudden cardiac death	CV (sudden death)	378	381	004 Baseline: FAP stage II, PND score II; NYHA class II, NT-proBNP 326.03 pmol/L, MLVWT 2.06cm Med. Hx: cardiomyopathy, congestive cardiac failure, mitral valve disease, atrial fibrillation, atrial

Patient Number Age/Gender Genotype	Cardiac Sub-group	Serious Adverse Event reported as Fatal	Adjudication (Subgroup)[a]	Study day(s) to Last dose of Study Drug	Study day (s) to Date of Death	Relevant Current and Historical Medical Condition
						flutter Patient had shortness of breath while climbing stairs and then collapsed. Sudden death with amyloidosis, restrictive cardiomyopathy, atrial flutter, malnutrition and anemia as contributory conditions.
[redacted] (b) (6) 42/F Ser50Arg	Yes	Cardiac failure, acute pulmonary edema	CV (sudden death)	356	378	004 Baseline: FAP stage II, PND score II; NYHA class II, NT-proBNP 503.39 pmol/L, MLVWT 1.87cm Med Hx: cardiomyopathy, cardiac failure Patient with 2 episodes respiratory arrest and cardiopulmonary resuscitation on same day. Cause of death listed as acute pulmonary edema secondary to heart failure and amyloidosis.
[redacted] (b) (6) 64/M Ser77Phe	No	Cardiac arrest	CV (presumed CV)	547	565	004 Baseline: FAP stage II, PND score IIIB; NYHA class II, NT-proBNP 1168.55 pmol/L, MLVWT 1.67cm Med Hx: atrial fibrillation, atrial flutter, hypertension, right bundle branch block During prolonged hospitalization for infected decubital ulcers, patient had cardiac arrest.
[redacted] (b) (6) 59/M Glu89Gln	Yes	Pulseless electrical activity	CV (sudden death)	169	172	004 Baseline: FAP stage II, PND score IIIA; NYHA class II, NT-proBNP 482.86 pmol/L, MLVWT 2.33cm Med Hx: cardiomyopathy, palpitations, left bundle branch block Prolonged episode of chest pain and palpitations at home; developed difficulty breathing and pulselessness. Resuscitation unsuccessful.
[redacted] (b) (6)	No	Cardiac failure	CV	526	529	004 Baseline: FAP stage II, PND score IIIB; NYHA

Patient Number Age/Gender Genotype	Cardiac Sub-group	Serious Adverse Event reported as Fatal	Adjudication (Subgroup)[a]	Study day(s) to Last dose of Study Drug	Study day (s) to Date of Death	Relevant Current and Historical Medical Condition
65/F Glu89Gln			(heart failure)			class II, MLVWT: 1.88cm; Med Hx: cardiac insufficiency, paroxysmal atrial fibrillation; hypotension, obstructive pulmonary disease, metabolic syndrome-hypercholesterolemia, obesity Worsening of cardiac insufficiency.
Placebo (n=6; 7.8%)						
(b) (6) 57/M V30M	No	Subarachnoid hemorrhage	CV (fatal stroke, hemorrhagic)	547	558	004 Baseline: FAP stage II, PND score IIIA; NYHA class I, NT-proBNP 11.56 pmol/L, MLVWT 1.21cm Med Hx: ischemic stroke (2007), right carotid stenosis, right retinal embolism, reactive hypertension, tobacco abuse Patient with sudden fall and cardio-respiratory arrest. Computed tomography of head showed subarachnoid hemorrhage
(b) (6) 61/M V30M	Yes	Staphylococcal sepsis	Non-CV	380	407	004 Baseline: FAP stage II, PND score IIIB; NYHA class II, NT-proBNP 416.42 pmol/L, MLVWT 1.98cm Med Hx: restrictive cardiomyopathy, congestive heart failure, pacemaker, hypotension Patient with cardiac arrest, and course complicated by cardiac failure, acute on chronic kidney injury, bacterial pneumonia, methicillin susceptible staphylococcus aureas septicemia and UTI. Multiple vegetations and erosions of cardiac valves consistent with staphylococcal endocarditis.
(b) (6)	Yes	Anemia, gastrointestinal	CV	338	422[b]	004 Baseline: FAP stage II, PND score IIIB; NYHA

Patient Number Age/Gender Genotype	Cardiac Sub-group	Serious Adverse Event reported as Fatal	Adjudication (Subgroup)[a]	Study day(s) to Last dose of Study Drug	Study day (s) to Date of Death	Relevant Current and Historical Medical Condition
58/M Thr59Lys		hemorrhage	(heart failure)			class I, NT-proBNP 600.74 pmol/L, MLVWT 1.63cm Med. Hx: restrictive cardiomyopathy, congestive heart failure, atrial fibrillation, pulmonary fibrosis, orthostatic hypotension, scleroderma, hypothyroidism Several hospitalizations for CHF exacerbations during study. Recent worsening of cardiac symptoms. Developed melena with anemia. Received transfusion 1 PRBC. Based on overall poor health and prognosis, transferred to palliative care.
(b) (6) [redacted] 43/M Ser52Pro	Yes	Acute kidney failure, urinary tract infection, bacteremia	Unknown	274	298	004 Baseline: FAP stage II, PND score IIIA; NYHA class I, NT-proBNP 69.38 pmol/L, MLVWT 1.32cm Med Hx: peripheral sensorimotor neuropathy UTI, acute renal failure (oliguria and anuria), and *E. coli* sepsis.
(b) (6) [redacted] 77/F Ala97Ser	Yes	Colorectal cancer metastatic	Non-CV	379	558[b]	004 Baseline: FAP stage II, PND score IIIB; NYHA class I, MLVWT 1.48cm Med Hx: colectomy for Stage 3 colon cancer in April 2013 (767 days before start in the study) Metastatic recurrence of colorectal cancer
(b) (6) [redacted] 56/F Glu89Gln	No	Ischemic stroke	CV (fatal stroke, ischemic)	108	134	004 Baseline: FAP stage II, PND score IIIA; NYHA class II, NT-proBNP 703.52 pmol/L, MLVWT 1.67 cm Med Hx: hypertension, hypotension, cardiomyopathy, atrial fibrillation, atrial flutter Ischemic stroke complicated by exacerbation of CHF, and pneumonia.

Patient Number Age/Gender Genotype	Cardiac Sub-group	Serious Adverse Event reported as Fatal	Adjudication (Subgroup)[a]	Study day(s) to Last dose of Study Drug	Study day (s) to Date of Death	Relevant Current and Historical Medical Condition
Study 003: Open-Label Extension Study						
(b) (6) [redacted] 75/F Val30Met	No	Esophageal carcinoma	Non-CV	568	656	003 Baseline: FAP stage I, PND score I; NHYA class I Med Hx: Atrioventricular block first degree, left bundle branch block, upper abdominal pain, irritable bowel syndrome, ankylosing spondylitis, asthma. Developed stomacb pain, worsening constipation, swallowing drfficulties. Endoscopy showed tumor at gastroesophageal junction. Computed tomography showed metastases.
(b) (6) [redacted] 75/M Val30Met	Yes	Myocardial infarction	CV (fatal MI)	735	757	003 Baseline: FAP stage II, PND score IIIA; NYHA class II, NT-proBNP 1758 ng/L, MLVWT 1.74 cm Med Hx: atrial fibrillation, hypertension, chronic renal disease, hx prostate cancer Had ECG changes and positive cardiac enzymes consistent with MI. Not a candidate for stent. Palliative care.
Study 006: Global Open-Label Extension Study						
004 Placebo/006 Patisiran-LNP Group						
(b) (6) [redacted] 71/M Val30Met	No	Cardiac Arrest	CV (sudden death)	64	82/649[c]	004 Baseline: FAP stage II, PND score IIIa; NYHA class II, NT-proBNP 69.50 pmol/L, MLVWT 2.04 cm 006 Baseline: FAP stage III, PND score IV; NYHA class II, NT-proBNP 360.84 pmol/L, MLVWT 1.94 cm Med Hx: AV block, restrictive cardiomyopathy,

Patient Number Age/Gender Genotype	Cardiac Sub-group	Serious Adverse Event reported as Fatal	Adjudication (Subgroup)[a]	Study day(s) to Last dose of Study Drug	Study day (s) to Date of Death	Relevant Current and Historical Medical Condition
						oropharyngeal squamous cell carcinoma, with dysphagia and weight loss Cardiac arrest: Patient found without vital signs, no treatment or resuscitation administered, no autopsy performed.
[redacted] (b) (6) 74/M Val30Met	Yes	Cardiac Arrest	CV (presumed CV death)	106	125/706[c]	004 Baseline: FAP stage II, PND score II; NYHA class I, NT-proBNP 438.84 pmol/L, MLVWT 1.59 cm 006 Baseline: FAP stage II, PND score IIIB; NYHA class I, NT-proBNP 256.41 pmol/L, MLVWT 1.51 cm Med Hx: heart failure, atrial fibrillation, pacemaker placement, chronic deep vein thrombosis, acute myelogenous leukemia Cardiac arrest: pulseless event in setting of multiple fentanyl IV injections (2 doses of 50 µg followed by 1 dose of 100 µg) for pain control of acute hip fracture
[redacted] (b) (6) 69/F Ile84Thr	No	Acute Respiratory Distress Syndrome Hemorrhagic Shock	Non- CV	106	111/678[c]	004 Baseline: FAP stage I, PND score II; NYHA class I, NT-proBNP 107.62 pmol/L, MLVWT 1.11 cm 006 Baseline: FAP stage II, PND score II; NYHA class I, NT-proBNP 157.65 pmol/L, MLVWT 1.23 cm Med Hx: cardiac amyloidosis, heart transplant (2008), hypertension, pulmonary edema, hypothyroidism, UTI, cyclosporine therapy, ARDS (parainfluenza), hemorrhagic shock Hemorrhagic shock was a fatal complication of
						ARDS: No autopsy performed, death certificate listed bleeding due to coagulopathy and respiratory failure due to parainfluenza as direct causes of death.
[redacted] (b) (6) 74/F Ala97Ser	Yes	Cardiogenic Shock	CV (presumed CV death)	22	39/605[c]	004 Baseline: FAP stage II, PND score IIIB; NYHA class II, NT-proBNP 102.90 pmol/L, MLVWT 1.52 cm 006 Baseline: FAP stage III, PND score IV; NYHA class III, NT-proBNP 211.69 pmol/L, MLVWT 1.37 cm Med Hx: cardiac failure, limbs edema, AV block-1, left bundle branch block, sensorimotor polyneuropathy, autonomic dysfunction Cardiogenic shock: Death occurred in a context of food aspiration, unknown if an autopsy was performed, death certificate stated cause was of natural origin and complication of amyloidosis
004 Patisiran-LNP/006 Patisiran-LNP Group						
[redacted] (b) (6) 78/F Thr60Ala	Yes	Invasive ductal breast carcinoma	Non-CV	19/590	262/832[b, c]	004 Baseline: FAP stage II, PND score IIIA; NYHA class II, NT-proBNP 282.73 pmol/L, MLVWT 1.98 cm 006 Baseline: FAP stage II, PND score IIIA; NYHA class II, NT-proBNP 526.99 pmol/L, MLVWT 1.69 cm Med Hx: atrial fibrillation; cardiac ablation, family history of breast cancer In Study 004, an undifferentiated right breast mass was identified. In Study 006, the diagnosis of invasive ductal breast carcinoma was confirmed. Patient withdrew from study and died 6.5 months

Patient Number Age/Gender Genotype	Cardiac Sub-group	Serious Adverse Event reported as Fatal	Adjudication (Subgroup)[a]	Study day(s) to Last dose of Study Drug	Study day (s) to Date of Death	Relevant Current and Historical Medical Condition
						after withdrawal from study
[redacted] (b) (6) 65/M Thr60Ala	Yes	Worsening Amyloidosis	CV (heart failure)	149/737	158/746[c]	004 Baseline: FAP stage II, PND score IIIA; NYHA class II, NT-proBNP 931.61 pmol/L; MLVWT 1.86 cm 006 Baseline: FAP stage III, PND score IV; NYHA class IV, NT-proBNP 726.64 pmol/L; MLVWT 1.66 cm Med Hx: hypothyroidism, QT prolongation Amyloidosis complication: death due to cardiopulmonary insufficiency, no autopsy completed; death certificate listed immediate cause of death as amyloidosis.

Abbreviations: ARDS=Adult respiratory distress syndrome; CHF=congestive heart failure; CV=Cardiovascular, ECG=electrocardiogram; F=female, FAP=familial amyloidosis polyneuropathy; M=male, Med Hx=medical history, MI=myocardial infarction; MLVWT=mean left ventricular wall thickness, NT-proBNP= B-type natriuretic peptide, NYHA=New York Heart Association, PND= polyneuropathy disability score; UTI=urinary tract infection.
[a]Definitions of classifications and sub-classifications are provided in the adjudication charter in CSR ALN-TTR02-004, Section 16.1.9.3.
[b] Patient off treatment for more than 30 days.
[c] Study day in Study 006/Study day for overall observation time from baseline in Study 004
Source: CSR ALN-TTR02-004: Table 14.3.2.1, Table 14.3.2.2, Appendix 16.2.4.2, Appendix 16.2.4.5.1, Appendix 16.2.4.5.2, Appendix 16.2.6.13, Appendix 16.2.6.14, Appendix 16.2.6.15, Appendix 16.2.7.7, Appendix 16.2.8.22, and Appendix 16.2.9.8; CSR ALN-TTR02-003: Appendix 16.2.1.1, Appendix 16.2.7.1, Appendix 16.2.4.3, Appendix 16.2.4.4; CSR ALN-TTR02-006: Table 14.3.2.1, Table 14.3.2.2, Appendix 16.2.7.7; ISS Table 3.2.1, Listing 1.

13.4. SAEs by System Organ Class, Study 004. Source: FDA Data Analysis

Tabulate

		TRT01A			
		Patisiran 0.3 mg/kg N=148		PLACEBO N=77	
AEBODSYS	AEDECOD	N Rows	Percent	N Rows	Percent
Cardiac disorders	Atrial fibrillation	2	1.35135%	1	1.29870%
	Cardiac amyloidosis	2	1.35135%	1	1.29870%
	Cardiac arrest	2	1.35135%	1	1.29870%
	Cardiac failure	3	2.02703%	2	2.59740%
	Cardiac failure congestive	3	2.02703%	2	2.59740%
	Conduction disorder	1	0.67568%	1	1.29870%
	Arteriosclerosis coronary artery	1	0.67568%	.	
	Atrioventricular block	1	0.67568%	.	
	Atrioventricular block complete	3	2.02703%	.	
	Cardiogenic shock	1	0.67568%	.	
	Cardiomyopathy	1	0.67568%	.	
	Pulseless electrical activity	1	0.67568%	.	
	Ventricular dyssynchrony	1	0.67568%	.	
	Ventricular fibrillation	1	0.67568%	.	
Gastrointestinal disorders	Diarrhoea	8	5.40541%	1	1.29870%
	Vomiting	1	0.67568%	3	3.89610%
	Ascites	1	0.67568%	.	
	Gastrointestinal motility disorder	1	0.67568%	.	
	Hiatus hernia	1	0.67568%	.	
	Impaired gastric emptying	1	0.67568%	.	
Infections and infestations	Erysipelas	1	0.67568%	1	1.29870%
	Pneumonia	3	2.02703%	3	3.89610%
	Bronchitis	1	0.67568%	.	
	Infected skin ulcer	1	0.67568%	.	
	Post procedural cellulitis	1	0.67568%	.	
	Urosepsis	1	0.67568%	.	
General disorders and administration site conditions	Chest discomfort	1	0.67568%	.	
	Device battery issue	1	0.67568%	.	
	Device dislocation	1	0.67568%	.	
	Influenza like illness	1	0.67568%	.	
	Oedema peripheral	1	0.67568%	.	
	Sudden cardiac death	1	0.67568%	.	
	Systemic inflammatory response syndro...	1	0.67568%	.	
Vascular disorders	Orthostatic hypotension	3	2.02703%	1	1.29870%
	Deep vein thrombosis	2	1.35135%	.	
	Hypotension	1	0.67568%	.	
	Peripheral arterial occlusive disease	1	0.67568%	.	
	Thrombophlebitis superficial	1	0.67568%	.	
Metabolism and nutrition disorders	Dehydration	1	0.67568%	3	3.89610%
	Cachexia	1	0.67568%	.	
	Hypocalcaemia	1	0.67568%	.	
	Hypoglycaemia	1	0.67568%	.	
Respiratory, thoracic and mediastinal disorders	Pleural effusion	1	0.67568%	1	1.29870%
	Acute pulmonary oedema	1	0.67568%	.	
	Dyspnoea	1	0.67568%	.	
	Respiratory failure	1	0.67568%	.	
Nervous system disorders	Ataxia	1	0.67568%	.	
	Cerebral infarction	1	0.67568%	.	
	Syncope	1	0.67568%	.	
	Transient ischaemic attack	1	0.67568%	.	
Eye disorders	Maculopathy	1	0.67568%	.	
	Vitreous haemorrhage	1	0.67568%	.	
	Vitreous opacities	1	0.67568%	.	
Injury, poisoning and procedural complications	Cervical vertebral fracture	1	0.67568%	.	
	Joint dislocation	1	0.67568%	.	
	Road traffic accident	1	0.67568%	.	
Musculoskeletal and connective tissue disorders	Back pain	1	0.67568%	.	
	Lumbar spinal stenosis	1	0.67568%	.	
	Neuropathic arthropathy	1	0.67568%	.	
Skin and subcutaneous tissue disorders	Skin ulcer	1	0.67568%	1	1.29870%
	Dermatitis	1	0.67568%	.	
Congenital, familial and genetic disorders	Phimosis	1	0.67568%	.	
	Syringomyelia	1	0.67568%	.	
Ear and labyrinth disorders	Deafness unilateral	1	0.67568%	.	
	Vertigo	1	0.67568%	.	
Investigations	Drug level increased	1	0.67568%	.	
	Investigation	1	0.67568%	.	
Neoplasms benign, malignant and unspecified (incl cysts and pol...	Atypical fibroxanthoma	1	0.67568%	.	
	Bladder cancer	1	0.67568%	.	
Renal and urinary disorders	Acute kidney injury	1	0.67568%	4	5.19481%
Blood and lymphatic system disorders	Anaemia of chronic disease	1	0.67568%	.	
Hepatobiliary disorders	Cholangitis	1	0.67568%	.	
Reproductive system and breast disorders	Breast mass	1	0.67568%	.	

13.5. Pooled SAEs Over All Studies. Source: ISS p. 796

Incidence of Serious Treatment-Emergent Adverse Events by System Organ Class and Preferred Term
Safety Population

System Organ Class Preferred Term	Patisiran 0.3 mg/kg 004 Pbo/ 006 Pati [1] (N=43)	004 Pati/ 006 Pati (N=148)	003 Pati/ 006 Pati (N=27)	Total (N=218)
At Least 1 Serious AE	13 (30.2) / 29	66 (44.6) / 136	9 (33.3) / 24	88 (40.4) / 189
Blood and lymphatic system disorders	0	1 (0.7) / 1	0	1 (0.5) / 1
Anaemia of chronic disease	0	1 (0.7) / 1	0	1 (0.5) / 1
Cardiac disorders	5 (11.6) / 5	29 (19.6) / 34	3 (11.1) / 3	37 (17.0) / 42
Arrhythmia	0	1 (0.7) / 1	0	1 (0.5) / 1
Arteriosclerosis coronary artery	0	1 (0.7) / 1	0	1 (0.5) / 1
Atrial fibrillation	0	2 (1.4) / 2	0	2 (0.9) / 2
Atrial tachycardia	0	1 (0.7) / 1	0	1 (0.5) / 1
Atrioventricular block	0	1 (0.7) / 1	0	1 (0.5) / 1
Atrioventricular block complete	0	3 (2.0) / 3	0	3 (1.4) / 3
Cardiac amyloidosis	0	4 (2.7) / 4	1 (3.7) / 1	5 (2.3) / 5
Cardiac arrest	2 (4.7) / 2	2 (1.4) / 2	0	4 (1.8) / 4
Cardiac failure	1 (2.3) / 1	4 (2.7) / 6	0	5 (2.3) / 7
Cardiac failure congestive	1 (2.3) / 1	4 (2.7) / 4	0	5 (2.3) / 5
Cardiogenic shock	1 (2.3) / 1	1 (0.7) / 1	0	2 (0.9) / 2
Cardiomyopathy	0	1 (0.7) / 1	0	1 (0.5) / 1
Conduction disorder	0	3 (2.0) / 3	0	3 (1.4) / 3
Myocardial infarction	0	0	1 (3.7) / 1	1 (0.5) / 1
Pulseless electrical activity	0	1 (0.7) / 1	0	1 (0.5) / 1
Restrictive cardiomyopathy	0	0	1 (3.7) / 1	1 (0.5) / 1
Sinus node dysfunction	0	1 (0.7) / 1	0	1 (0.5) / 1
Ventricular dyssynchrony	0	1 (0.7) / 1	0	1 (0.5) / 1
Ventricular fibrillation	0	1 (0.7) / 1	0	1 (0.5) / 1
Congenital, familial and genetic disorders	0	2 (1.4) / 2	0	2 (0.9) / 2
Phimosis	0	1 (0.7) / 1	0	1 (0.5) / 1
Syringomyelia	0	1 (0.7) / 1	0	1 (0.5) / 1
Ear and labyrinth disorders	0	4 (2.7) / 4	0	4 (1.8) / 4
Deafness unilateral	0	1 (0.7) / 1	0	1 (0.5) / 1
Sudden hearing loss	0	1 (0.7) / 1	0	1 (0.5) / 1
Vertigo	0	1 (0.7) / 1	0	1 (0.5) / 1

Note: Data are presented as the number of patients (%)[2]/number of events[3].
[1] Patisiran experience for patients who received placebo in Study 004 and were newly dosed with patisiran in Study 006.
[2] If a patient experienced more than one event in a given SOC or PT, the patient is counted once for that SOC or PT, respectively.
[3] The total number of events for all patients; a patient can be counted more than once if the patient has multiple events.

System Organ Class Preferred Term	Patisiran 0.3 mg/kg 004 Pbo/ 006 Pati [1] (N=43)	 004 Pati/ 006 Pati (N=148)	 003 Pati/ 006 Pati (N=27)	 Total (N=218)
Vertigo positional	0	1 (0.7) / 1	0	1 (0.5) / 1
Endocrine disorders	1 (2.3) / 1	0	0	1 (0.5) / 1
Hyperthyroidism	1 (2.3) / 1	0	0	1 (0.5) / 1
Eye disorders	0	3 (2.0) / 3	0	3 (1.4) / 3
Maculopathy	0	1 (0.7) / 1	0	1 (0.5) / 1
Vitreous haemorrhage	0	1 (0.7) / 1	0	1 (0.5) / 1
Vitreous opacities	0	1 (0.7) / 1	0	1 (0.5) / 1
Gastrointestinal disorders	3 (7.0) / 5	13 (8.8) / 14	0	16 (7.3) / 19
Ascites	0	1 (0.7) / 1	0	1 (0.5) / 1
Diarrhoea	1 (2.3) / 1	8 (5.4) / 9	0	9 (4.1) / 10
Faeces pale	1 (2.3) / 1	0	0	1 (0.5) / 1
Gastrointestinal motility disorder	0	1 (0.7) / 1	0	1 (0.5) / 1
Hiatus hernia	0	1 (0.7) / 1	0	1 (0.5) / 1
Ileus	1 (2.3) / 1	0	0	1 (0.5) / 1
Impaired gastric emptying	0	1 (0.7) / 1	0	1 (0.5) / 1
Nausea	1 (2.3) / 1	0	0	1 (0.5) / 1
Vomiting	1 (2.3) / 1	1 (0.7) / 1	0	2 (0.9) / 2
General disorders and administration site conditions	1 (2.3) / 1	8 (5.4) / 10	1 (3.7) / 1	10 (4.6) / 12
Asthenia	1 (2.3) / 1	2 (1.4) / 2	0	3 (1.4) / 3
Chest discomfort	0	1 (0.7) / 1	1 (3.7) / 1	2 (0.9) / 2
Device battery issue	0	1 (0.7) / 1	0	1 (0.5) / 1
Device dislocation	0	1 (0.7) / 2	0	1 (0.5) / 2
Influenza like illness	0	1 (0.7) / 1	0	1 (0.5) / 1
Oedema peripheral	0	1 (0.7) / 1	0	1 (0.5) / 1
Sudden cardiac death	0	1 (0.7) / 1	0	1 (0.5) / 1
Systemic inflammatory response syndrome	0	1 (0.7) / 1	0	1 (0.5) / 1
Hepatobiliary disorders	0	1 (0.7) / 1	0	1 (0.5) / 1
Cholangitis	0	1 (0.7) / 1	0	1 (0.5) / 1
Immune system disorders	0	1 (0.7) / 1	0	1 (0.5) / 1
Amyloidosis	0	1 (0.7) / 1	0	1 (0.5) / 1
Infections and infestations	2 (4.7) / 2	10 (6.8) / 11	2 (7.4) / 3	14 (6.4) / 16
Abscess limb	0	0	1 (3.7) / 1	1 (0.5) / 1
Bronchitis	0	1 (0.7) / 1	0	1 (0.5) / 1
Cellulitis	1 (2.3) / 1	1 (0.7) / 1	0	2 (0.9) / 2
Erysipelas	0	1 (0.7) / 1	0	1 (0.5) / 1
Infected skin ulcer	0	1 (0.7) / 2	0	1 (0.5) / 2
Osteomyelitis	0	0	1 (3.7) / 1	1 (0.5) / 1
Pneumonia	0	4 (2.7) / 4	0	4 (1.8) / 4
Post procedural cellulitis	0	1 (0.7) / 1	0	1 (0.5) / 1
Urinary tract infection	1 (2.3) / 1	0	1 (3.7) / 1	2 (0.9) / 2
Urosepsis	0	1 (0.7) / 1	0	1 (0.5) / 1
Injury, poisoning and procedural complications	2 (4.7) / 2	6 (4.1) / 8	2 (7.4) / 7	10 (4.6) / 17
Ankle fracture	0	0	1 (3.7) / 1	1 (0.5) / 1
Burns second degree	0	1 (0.7) / 1	0	1 (0.5) / 1
Cervical vertebral fracture	0	1 (0.7) / 1	0	1 (0.5) / 1

System Organ Class Preferred Term	Patisiran 0.3 mg/kg 004 Pbo/ 006 Pati [1] (N=43)	 004 Pati/ 006 Pati (N=148)	 003 Pati/ 006 Pati (N=27)	 Total (N=218)
Femur fracture	0	1 (0.7) / 1	1 (3.7) / 1	2 (0.9) / 2
Foot fracture	0	0	1 (3.7) / 1	1 (0.5) / 1
Hip fracture	1 (2.3) / 1	1 (0.7) / 1	0	2 (0.9) / 2
Joint dislocation	0	1 (0.7) / 2	0	1 (0.5) / 2
Ligament rupture	0	0	1 (3.7) / 1	1 (0.5) / 1
Lower limb fracture	0	1 (0.7) / 1	0	1 (0.5) / 1
Road traffic accident	0	1 (0.7) / 1	0	1 (0.5) / 1
Spinal compression fracture	1 (2.3) / 1	0	0	1 (0.5) / 1
Thermal burn	0	0	1 (3.7) / 1	1 (0.5) / 1
Tibia fracture	0	0	1 (3.7) / 2	1 (0.5) / 2
Investigations	0	3 (2.0) / 3	0	3 (1.4) / 3
Drug level increased	0	1 (0.7) / 1	0	1 (0.5) / 1
Investigation	0	1 (0.7) / 1	0	1 (0.5) / 1
Transaminases increased	0	1 (0.7) / 1	0	1 (0.5) / 1
Metabolism and nutrition disorders	1 (2.3) / 2	4 (2.7) / 4	1 (3.7) / 2	6 (2.8) / 8
Cachexia	0	1 (0.7) / 1	0	1 (0.5) / 1
Dehydration	0	1 (0.7) / 1	1 (3.7) / 2	2 (0.9) / 3
Fluid overload	1 (2.3) / 1	0	0	1 (0.5) / 1
Hypocalcaemia	0	1 (0.7) / 1	0	1 (0.5) / 1
Hypoglycaemia	0	1 (0.7) / 1	0	1 (0.5) / 1
Hypokalaemia	1 (2.3) / 1	0	0	1 (0.5) / 1
Musculoskeletal and connective tissue disorders	1 (2.3) / 1	3 (2.0) / 3	2 (7.4) / 2	6 (2.8) / 6
Back pain	0	1 (0.7) / 1	0	1 (0.5) / 1
Lumbar spinal stenosis	0	1 (0.7) / 1	0	1 (0.5) / 1
Neuropathic arthropathy	0	1 (0.7) / 1	0	1 (0.5) / 1
Osteoarthritis	1 (2.3) / 1	0	0	1 (0.5) / 1
Osteonecrosis	0	0	2 (7.4) / 2	2 (0.9) / 2
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1 (2.3) / 1	5 (3.4) / 9	1 (3.7) / 1	7 (3.2) / 11
Atypical fibroxanthoma	0	1 (0.7) / 2	0	1 (0.5) / 2
Bladder cancer	0	1 (0.7) / 2	0	1 (0.5) / 2
Bladder cancer recurrent	0	1 (0.7) / 2	0	1 (0.5) / 2
Gastric cancer	0	1 (0.7) / 1	0	1 (0.5) / 1
Hypopharyngeal cancer	1 (2.3) / 1	0	0	1 (0.5) / 1
Intraductal proliferative breast lesion	0	1 (0.7) / 1	0	1 (0.5) / 1
Invasive ductal breast carcinoma	0	1 (0.7) / 1	0	1 (0.5) / 1
Oesophageal carcinoma	0	0	1 (3.7) / 1	1 (0.5) / 1
Nervous system disorders	2 (4.7) / 2	7 (4.7) / 8	1 (3.7) / 1	10 (4.6) / 11
Ataxia	0	1 (0.7) / 1	0	1 (0.5) / 1
Autonomic nervous system imbalance	0	1 (0.7) / 1	0	1 (0.5) / 1
Cerebral infarction	0	1 (0.7) / 2	0	1 (0.5) / 2
Cerebrovascular accident	0	0	1 (3.7) / 1	1 (0.5) / 1
Dizziness	1 (2.3) / 1	0	0	1 (0.5) / 1
Lethargy	0	1 (0.7) / 1	0	1 (0.5) / 1
Syncope	1 (2.3) / 1	2 (1.4) / 2	0	3 (1.4) / 3
Transient ischaemic attack	0	1 (0.7) / 1	0	1 (0.5) / 1

	Patisiran 0.3 mg/kg			
System Organ Class Preferred Term	004 Pbo/ 006 Pati [1] (N=43)	004 Pati/ 006 Pati (N=148)	003 Pati/ 006 Pati (N=27)	Total (N=218)
Renal and urinary disorders	2 (4.7) / 2	2 (1.4) / 2	1 (3.7) / 2	5 (2.3) / 6
Acute kidney injury	0	1 (0.7) / 1	0	1 (0.5) / 1
Acute prerenal failure	0	0	1 (3.7) / 2	1 (0.5) / 2
Chronic kidney disease	1 (2.3) / 1	1 (0.7) / 1	0	2 (0.9) / 2
Urinary retention	1 (2.3) / 1	0	0	1 (0.5) / 1
Reproductive system and breast disorders	0	1 (0.7) / 1	0	1 (0.5) / 1
Breast mass	0	1 (0.7) / 1	0	1 (0.5) / 1
Respiratory, thoracic and mediastinal disorders	1 (2.3) / 1	5 (3.4) / 6	0	6 (2.8) / 7
Acute pulmonary oedema	0	1 (0.7) / 1	0	1 (0.5) / 1
Acute respiratory distress syndrome	1 (2.3) / 1	0	0	1 (0.5) / 1
Dyspnoea	0	1 (0.7) / 1	0	1 (0.5) / 1
Pleural effusion	0	1 (0.7) / 1	0	1 (0.5) / 1
Respiratory failure	0	1 (0.7) / 1	0	1 (0.5) / 1
Sleep apnoea syndrome	0	1 (0.7) / 2	0	1 (0.5) / 2
Skin and subcutaneous tissue disorders	1 (2.3) / 1	2 (1.4) / 2	0	3 (1.4) / 3
Decubitus ulcer	1 (2.3) / 1	0	0	1 (0.5) / 1
Dermatitis	0	1 (0.7) / 1	0	1 (0.5) / 1
Skin ulcer	0	1 (0.7) / 1	0	1 (0.5) / 1
Surgical and medical procedures	0	0	1 (3.7) / 1	1 (0.5) / 1
Arthrodesis	0	0	1 (3.7) / 1	1 (0.5) / 1
Vascular disorders	2 (4.7) / 3	9 (6.1) / 9	1 (3.7) / 1	12 (5.5) / 13
Deep vein thrombosis	0	2 (1.4) / 2	0	2 (0.9) / 2
Hypotension	1 (2.3) / 1	1 (0.7) / 1	0	2 (0.9) / 2
Orthostatic hypotension	0	4 (2.7) / 4	0	4 (1.8) / 4
Peripheral arterial occlusive disease	0	1 (0.7) / 1	0	1 (0.5) / 1
Phlebitis	1 (2.3) / 1	0	0	1 (0.5) / 1
Shock haemorrhagic	1 (2.3) / 1	0	0	1 (0.5) / 1
Thrombophlebitis superficial	0	1 (0.7) / 1	0	1 (0.5) / 1
Venous thrombosis limb	0	0	1 (3.7) / 1	1 (0.5) / 1

13.6. AEs Leading to Discontinuation/Withdrawal from Study 004. Source: Study 004 CSR

Incidence of Treatment-Emergent Adverse Events Leading to Treatment Discontinuation by System Organ Class and Preferred Term (Safety Population)

System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
At Least 1 AE	11 (14.3)/15	7 (4.7)/14
Blood and lymphatic system disorders	1 (1.3)/1	0
Iron deficiency anaemia	1 (1.3)/1	0
Cardiac disorders	1 (1.3)/1	4 (2.7)/4
Cardiac arrest	0	1 (0.7)/1
Cardiac failure	1 (1.3)/1	2 (1.4)/2
Pulseless electrical activity	0	1 (0.7)/1
Gastrointestinal disorders	0	1 (0.7)/2
Dry mouth	0	1 (0.7)/1
Dysphagia	0	1 (0.7)/1
General disorders and administration site conditions	1 (1.3)/1	1 (0.7)/1
General physical health deterioration	1 (1.3)/1	0
Sudden cardiac death	0	1 (0.7)/1
Immune system disorders	1 (1.3)/1	1 (0.7)/1
Amyloidosis	1 (1.3)/1	0
Infusion related reaction	0	1 (0.7)/1

[1] If a patient experienced more than 1 event in a given SOC, that patient is counted once for the SOC. If a patient experienced more than 1 event with a given PT, that patient is counted only once for that PT.
[2] The total number of events for all patients; a patient can be counted more than once if the patient has multiple events.
The Medical Dictionary for Regulatory Activities (MedDRA) version 18.0 is used to code adverse events.
TEAEs are those with onset during or after the first dose through 28 days following the last dose of study drug. In addition, any event that was present at baseline but worsened in intensity or was subsequently considered drug-related is considered a TEAE.

System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Infections and infestations	2 (2.6)/3	0
Bacteraemia	1 (1.3)/1	0
Staphylococcal sepsis	1 (1.3)/1	0
Urinary tract infection	1 (1.3)/1	0
Musculoskeletal and connective tissue disorders	0	1 (0.7)/1
Muscular weakness	0	1 (0.7)/1
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	2 (2.6)/2	0
Colon cancer metastatic	1 (1.3)/1	0
Colorectal cancer metastatic	1 (1.3)/1	0
Nervous system disorders	3 (3.9)/3	1 (0.7)/3
Dysgeusia	0	1 (0.7)/1
Hyperaesthesia	0	1 (0.7)/1
Hypoaesthesia	0	1 (0.7)/1
Ischaemic stroke	1 (1.3)/1	0
Neuropathy peripheral	1 (1.3)/1	0
Posterior reversible encephalopathy syndrome	1 (1.3)/1	0
Renal and urinary disorders	2 (2.6)/2	0
Acute kidney injury	2 (2.6)/2	0

System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Respiratory, thoracic and mediastinal disorders	0	1 (0.7)/1
Acute pulmonary oedema	0	1 (0.7)/1
Skin and subcutaneous tissue disorders	0	1 (0.7)/1
Skin atrophy	0	1 (0.7)/1
Vascular disorders	1 (1.3)/1	0
Peripheral arterial occlusive disease	1 (1.3)/1	0

13.7. AEs Leading to Discontinuation/Withdrawal: Pooled Over All Studies. Source: ISS, p. 819

Incidence of Treatment-Emergent Adverse Events Leading to Treatment Discontinuation by System Organ Class and Preferred Term
Safety Population

System Organ Class Preferred Term	Patisiran 0.3 mg/kg 004 Pbo/ 006 Pati [1] (N=43)	004 Pati/ 006 Pati (N=148)	003 Pati/ 006 Pati (N=27)	Total (N=218)
At Least 1 AE Leading to Treatment Discontinuation	1 (2.3) / 1	12 (8.1) / 20	0	13 (6.0) / 21
Cardiac disorders	0	5 (3.4) / 5	0	5 (2.3) / 5
Cardiac arrest	0	1 (0.7) / 1	0	1 (0.5) / 1
Cardiac failure	0	2 (1.4) / 2	0	2 (0.9) / 2
Cardiac failure congestive	0	1 (0.7) / 1	0	1 (0.5) / 1
Pulseless electrical activity	0	1 (0.7) / 1	0	1 (0.5) / 1
Gastrointestinal disorders	0	1 (0.7) / 2	0	1 (0.5) / 2
Dry mouth	0	1 (0.7) / 1	0	1 (0.5) / 1
Dysphagia	0	1 (0.7) / 1	0	1 (0.5) / 1
General disorders and administration site conditions	0	2 (1.4) / 2	0	2 (0.9) / 2
Asthenia	0	1 (0.7) / 1	0	1 (0.5) / 1
Sudden cardiac death	0	1 (0.7) / 1	0	1 (0.5) / 1
Immune system disorders	0	1 (0.7) / 1	0	1 (0.5) / 1

Note: This table summarizes all TEAEs leading to study drug discontinuation as indicated on the AE case report form.
Note: Data are presented as the number of patients (%)[2]/number of events[3].
[1] Patisiran experience for patients who received placebo in Study 004 and were newly dosed with patisiran in Study 006.
[2] If a patient experienced more than one event in a given SOC or PT, the patient is counted once for that SOC or PT, respectively.
[3] The total number of events for all patients; a patient can be counted more than once if the patient has multiple events.

Incidence of Treatment-Emergent Adverse Events Leading to Treatment Discontinuation by System Organ Class and Preferred Term
Safety Population

	Patisiran 0.3 mg/kg			
System Organ Class Preferred Term	004 Pbo/ 006 Pati [1] (N=43)	004 Pati/ 006 Pati (N=148)	003 Pati/ 006 Pati (N=27)	Total (N=218)
Infusion related reaction	0	1 (0.7) / 1	0	1 (0.5) / 1
Musculoskeletal and connective tissue disorders	0	1 (0.7) / 1	0	1 (0.5) / 1
Muscular weakness	0	1 (0.7) / 1	0	1 (0.5) / 1
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0	3 (2.0) / 3	0	3 (1.4) / 3
Gastric cancer	0	1 (0.7) / 1	0	1 (0.5) / 1
Intraductal proliferative breast lesion	0	1 (0.7) / 1	0	1 (0.5) / 1
Invasive ductal breast carcinoma	0	1 (0.7) / 1	0	1 (0.5) / 1
Nervous system disorders	0	1 (0.7) / 3	0	1 (0.5) / 3
Dysgeusia	0	1 (0.7) / 1	0	1 (0.5) / 1
Hyperaesthesia	0	1 (0.7) / 1	0	1 (0.5) / 1
Hypoaesthesia	0	1 (0.7) / 1	0	1 (0.5) / 1
Renal and urinary disorders	1 (2.3) / 1	1 (0.7) / 1	0	2 (0.9) / 2
Chronic kidney disease	1 (2.3) / 1	1 (0.7) / 1	0	2 (0.9) / 2

Incidence of Treatment-Emergent Adverse Events Leading to Treatment Discontinuation by System Organ Class and Preferred Term
Safety Population

	Patisiran 0.3 mg/kg			
System Organ Class Preferred Term	004 Pbo/ 006 Pati [1] (N=43)	004 Pati/ 006 Pati (N=148)	003 Pati/ 006 Pati (N=27)	Total (N=218)
Respiratory, thoracic and mediastinal disorders	0	1 (0.7) / 1	0	1 (0.5) / 1
Acute pulmonary oedema	0	1 (0.7) / 1	0	1 (0.5) / 1
Skin and subcutaneous tissue disorders	0	1 (0.7) / 1	0	1 (0.5) / 1
Skin atrophy	0	1 (0.7) / 1	0	1 (0.5) / 1

13.8. AEs by Severity, Study 004. Excerpt from Study 004 CSR, p. 872

Incidence of Treatment-Emergent Adverse Events by Maximum Severity by System Organ Class
and Preferred Term (Safety Population)

System Organ Class/ Preferred Term	Statistic	Placebo (N=77)			Patisiran 0.3 mg/kg (N=148)		
		Mild	Moderate	Severe	Mild	Moderate	Severe
At Least 1 AE	N (%)	16 (20.8)	31 (40.3)	28 (36.4)	35 (23.6)	66 (44.6)	42 (28.4)
Blood and lymphatic system disorders	N (%)	7 (9.1)	3 (3.9)	2 (2.6)	6 (4.1)	2 (1.4)	1 (0.7)
Anaemia	N (%)	5 (6.5)	1 (1.3)	2 (2.6)	3 (2.0)	0	0
Anaemia macrocytic	N (%)	0	0	0	0	1 (0.7)	0
Anaemia of chronic disease	N (%)	0	0	0	0	1 (0.7)	0
Iron deficiency anaemia	N (%)	0	1 (1.3)	0	0	0	0
Leukocytosis	N (%)	1 (1.3)	1 (1.3)	0	0	0	0
Leukopenia	N (%)	1 (1.3)	0	0	0	0	1 (0.7)
Microcytic anaemia	N (%)	0	0	0	1 (0.7)	0	0
Normochromic normocytic anaemia	N (%)	1 (1.3)	0	0	0	0	0
Spontaneous haematoma	N (%)	1 (1.3)	0	0	3 (2.0)	0	0
Thrombocytopenia	N (%)	0	1 (1.3)	0	1 (0.7)	0	0
Cardiac disorders	N (%)	14 (18.2)	8 (10.4)	6 (7.8)	17 (11.5)	12 (8.1)	13 (8.8)
Arteriosclerosis coronary artery	N (%)	0	0	0	1 (0.7)	0	0
Atrial fibrillation	N (%)	1 (1.3)	4 (5.2)	0	5 (3.4)	7 (4.7)	1 (0.7)
Atrial flutter	N (%)	0	0	0	0	2 (1.4)	0
Atrioventricular block	N (%)	0	0	0	0	0	1 (0.7)
Atrioventricular block complete	N (%)	0	0	0	0	0	3 (2.0)
Atrioventricular block first degree	N (%)	4 (5.2)	0	0	0	0	0
Atrioventricular block second degree	N (%)	0	0	0	1 (0.7)	0	0
Bradycardia	N (%)	0	0	0	2 (1.4)	1 (0.7)	0
Bundle branch block left	N (%)	0	0	0	2 (1.4)	0	0

Note: Patients who report multiple occurrences of the same adverse event (preferred term) are classified according to the highest severity. Patients are therefore included only once per body system and preferred term. Severe AEs include both severe events and events with missing severity. The Medical Dictionary for Regulatory Activities (MedDRA) version 18.0 is used to code adverse events. TEAEs are those with onset during or after the first dose through 28 days following the last dose of study drug. In addition, any event that was present at baseline but worsened in intensity or was subsequently considered drug-related is considered a TEAE.

Incidence of Treatment-Emergent Adverse Events by Maximum Severity by System Organ Class
and Preferred Term (Safety Population)

System Organ Class/ Preferred Term	Statistic	Placebo (N=77)			Patisiran 0.3 mg/kg (N=148)		
		Mild	Moderate	Severe	Mild	Moderate	Severe
Bundle branch block right	N (%)	2 (2.6)	0	0	3 (2.0)	0	0
Cardiac amyloidosis	N (%)	1 (1.3)	0	0	1 (0.7)	3 (2.0)	0
Cardiac arrest	N (%)	0	1 (1.3)	0	0	0	2 (1.4)
Cardiac failure	N (%)	1 (1.3)	1 (1.3)	2 (2.6)	2 (1.4)	2 (1.4)	3 (2.0)
Cardiac failure acute	N (%)	0	1 (1.3)	0	0	0	0
Cardiac failure chronic	N (%)	0	0	1 (1.3)	0	0	0
Cardiac failure congestive	N (%)	0	1 (1.3)	1 (1.3)	0	4 (2.7)	1 (0.7)
Cardio-respiratory arrest	N (%)	0	0	1 (1.3)	0	0	0
Cardiogenic shock	N (%)	0	0	0	0	0	1 (0.7)
Cardiomegaly	N (%)	0	0	0	0	0	1 (0.7)
Cardiomyopathy	N (%)	0	0	0	1 (0.7)	0	0
Conduction disorder	N (%)	0	1 (1.3)	0	0	1 (0.7)	0
Cyanosis	N (%)	1 (1.3)	0	0	0	0	0
Palpitations	N (%)	2 (2.6)	0	0	1 (0.7)	0	0
Pulseless electrical activity	N (%)	0	0	0	0	0	1 (0.7)
Restrictive cardiomyopathy	N (%)	0	0	1 (1.3)	0	0	0
Right ventricular failure	N (%)	0	0	0	1 (0.7)	0	0
Sinus bradycardia	N (%)	1 (1.3)	0	0	0	0	0
Sinus node dysfunction	N (%)	0	0	0	0	1 (0.7)	0
Sinus tachycardia	N (%)	1 (1.3)	0	0	0	0	0
Supraventricular extrasystoles	N (%)	5 (6.5)	0	0	2 (1.4)	0	0
Supraventricular tachycardia	N (%)	0	1 (1.3)	0	0	0	0
Tachyarrhythmia	N (%)	0	0	0	1 (0.7)	0	0
Tachycardia	N (%)	0	0	0	1 (0.7)	0	0

Incidence of Treatment-Emergent Adverse Events by Maximum Severity by System Organ Class and Preferred Term (Safety Population)

System Organ Class/ Preferred Term	Statistic	Placebo (N=77) Mild	Placebo (N=77) Moderate	Placebo (N=77) Severe	Patisiran 0.3 mg/kg (N=148) Mild	Patisiran 0.3 mg/kg (N=148) Moderate	Patisiran 0.3 mg/kg (N=148) Severe
Trifascicular block	N (%)	0	0	0	1 (0.7)	0	0
Ventricular dyssynchrony	N (%)	0	0	0	0	0	1 (0.7)
Ventricular extrasystoles	N (%)	2 (2.6)	0	0	0	0	0
Ventricular fibrillation	N (%)	0	0	0	0	0	1 (0.7)
Ventricular tachycardia	N (%)	0	2 (2.6)	0	0	0	0
Congenital, familial and genetic disorders	N (%)	2 (2.6)	1 (1.3)	0	1 (0.7)	1 (0.7)	1 (0.7)
Hereditary neuropathic amyloidosis	N (%)	1 (1.3)	1 (1.3)	0	0	0	0
Hypertrophic cardiomyopathy	N (%)	1 (1.3)	0	0	0	1 (0.7)	0
Phimosis	N (%)	0	0	0	1 (0.7)	0	0
Syringomyelia	N (%)	0	0	0	0	0	1 (0.7)
Ear and labyrinth disorders	N (%)	1 (1.3)	1 (1.3)	0	6 (4.1)	6 (4.1)	2 (1.4)
Deafness unilateral	N (%)	0	0	0	0	1 (0.7)	1 (0.7)
Ear pain	N (%)	0	0	0	0	1 (0.7)	0
Eustachian tube disorder	N (%)	0	0	0	1 (0.7)	0	0
Hypoacusis	N (%)	0	0	0	1 (0.7)	1 (0.7)	0
Otorrhoea	N (%)	1 (1.3)	0	0	0	0	0
Sudden hearing loss	N (%)	0	0	0	0	1 (0.7)	0
Tinnitus	N (%)	0	0	0	2 (1.4)	1 (0.7)	0
Vertigo	N (%)	0	1 (1.3)	0	5 (3.4)	2 (1.4)	1 (0.7)
Endocrine disorders	N (%)	2 (2.6)	1 (1.3)	0	1 (0.7)	0	0

13.9. AEs in Study 004. Source: FDA Data Analysis

		TRT01A			
		Patisiran 0.3 mg/kg		PLACEBO	
		N=148		N=77	
AEBODSYS	**AEDECOD**	**N Rows**	**Percent**	**N Rows**	**Percent**
General disorders and administration site conditions	Abdominal pain, Distension, Bloating, Spasm, Ibs, Megacolon	3	2.0%	1	1.3%
	Chest discomfort	6	4.1%	2	2.6%
	Chills	3	2.0%	1	1.3%
	Feeling hot	6	4.1%	1	1.3%
	Infusion site extravasation	4	2.7%	1	1.3%
	Oedema peripheral	44	29.7%	17	22.1%
	Chest pain (non-cardiac or unknown)	3	2.0%	.	.
	Drug intolerance	4	2.7%	.	.
	Injection site erythema	3	2.0%	.	.
Skin and subcutaneous tissue disorders	Blister	3	2.0%	1	1.3%
	Dermatitis	3	2.0%	1	1.3%
	Eczema	6	4.1%	1	1.3%
	Erythema	11	7.4%	2	2.6%

		TRT01A			
		Patisiran 0.3 mg/kg		PLACEBO	
		N=148		N=77	
AEBODSYS	**AEDECOD**	**N Rows**	**Percent**	**N Rows**	**Percent**
	Hyperhidrosis	4	2.7%	1	1.3%
	Night sweats	4	2.7%	1	1.3%
	Pruritus	5	3.4%	2	2.6%
	Skin discolouration	3	2.0%	.	.
Gastrointestinal disorders	Abdominal pain, Distension, Bloating, Spasm, Ibs, Megacolon	7	4.7%	1	1.3%
	Abdominal distension	4	2.7%	1	1.3%
	Dental caries	4	2.7%	1	1.3%
	Dry mouth, Dry lips, Thirst	7	4.7%	3	3.9%
	Dyspepsia, N, V, Indigestion, Epigastric pain, Gastritis, Duoden	14	9.5%	6	7.8%
	Dysphagia	4	2.7%	2	2.6%
	Toothache	4	2.7%	1	1.3%
Nervous system disorders	Fall, Dizziness, Balance disorder, Gait disturbance, Difficulty walking	27	18.2%	13	16.9%
	Neuralgia, Neuritis, Neuropathy	10	6.8%	5	6.5%
	Paraesthesia	8	5.4%	3	3.9%
	Restless legs syndrome	4	2.7%	2	2.6%
	Visual field defect	7	4.7%	3	3.9%
	Burning sensation	3	2.0%	.	.
	Muscle contractions involuntary	3	2.0%	.	.
	Radicular pain	3	2.0%	.	.
	Tension headache	3	2.0%	.	.
Infections and infestations	Bronchitis, Bronchiolitis, Tracheitis, Alveolitis, Bronchiectasis	10	6.8%	2	2.6%
	Influenza	11	7.4%	4	5.2%
	Nasopharyngitis	15	10.1%	6	7.8%
	Pharyngitis	5	3.4%	1	1.3%
	Upper respiratory tract infection	13	8.8%	5	6.5%
	Sinusitis	6	4.1%	.	.
	Uri, Cold, Rhinitis, Upper resp tract infection, Flu-Like illne	6	4.1%	.	.
Musculoskeletal and connective tissue disorders	Back pain	20	13.5%	6	7.8%
	Muscle spasms	13	8.8%	1	1.3%
	Musculoskeletal chest pain	5	3.4%	1	1.3%
	Osteopenia	4	2.7%	1	1.3%
	Arthralgia, Arthritis, Arthrosis	13	8.8%	.	.
	Joint stiffness	5	3.4%	.	.
Injury, poisoning and procedural complications	Burns second degree	4	2.7%	1	1.3%

		TRT01A			
		Patisiran 0.3 mg/kg		PLACEBO	
		N=148		N=77	
AEBODSYS	**AEDECOD**	**N Rows**	**Percent**	**N Rows**	**Percent**
	Ligament sprain	5	3.4%	2	2.6%
	Limb injury	3	2.0%	1	1.3%
	Post-Traumatic pain	3	2.0%	1	1.3%
	Skin abrasion	3	2.0%	.	.
Cardiac disorders	Atrial fibrillation	13	8.8%	5	6.5%
	Cardiac amyloidosis	4	2.7%	1	1.3%
	Cardiac failure congestive	5	3.4%	2	2.6%
	Atrioventricular block complete	3	2.0%	.	.
	Bradycardia	3	2.0%	.	.
Eye disorders	Dry eye	7	4.7%	2	2.6%
	Vision blurred	4	2.7%	1	1.3%
	Vitreous floaters	3	2.0%	1	1.3%
Vascular disorders	Hot flush	3	2.0%	1	1.3%
	Hypertension, Bp increased	6	4.1%	2	2.6%
	Hypotension	10	6.8%	5	6.5%
Respiratory, thoracic and mediastinal disorders	Dysphonia	4	2.7%	.	.
	Dyspnoea	13	8.8%	.	.
	Dyspnoea exertional	3	2.0%	.	.
	Sleep apnoea syndrome	3	2.0%	.	.
Ear and labyrinth disorders	Vertigo; vestibular dysfunction	8	5.4%	1	1.3%
	Tinnitus	3	2.0%	.	.
Investigations	Blood creatinine increased	3	2.0%	.	.
	Creatinine renal clearance decreased	4	2.7%	.	.
	Weight increased	4	2.7%	.	.
Blood and lymphatic system disorders	Spontaneous haematoma	3	2.0%	1	1.3%
Immune system disorders	Infusion related reaction	28	18.9%	7	9.1%
Metabolism and nutrition disorders	Hypoglycaemia	4	2.7%	.	.
	Iron deficiency	3	2.0%	.	.
Psychiatric disorders	Insomnia, Sleep disturbance, Abnormal dreams	15	10.1%	7	9.1%

13.10. Subjects with Renal Abnormalities in Study 004

The following patient summaries are copied from the Study 004 CSR, p. 249.

<u>Patisiran-LNP group</u>

<u>Patisiran-LNP patients with creatinine value of >3 × baseline or >4 mg/dL and a concurrent eGFR of <15 mL/min/1.73 m²</u>

Patient [redacted] (b) (6), a 60-year-old white male with history of atrial fibrillation, had a low creatinine of 53 µmol/L and normal eGFR of 137.6 mL/min/1.73 m^2 at baseline. On Day 84, the patient had an elevated creatinine of 415 µmol/L (Grade 3) and eGFR of 12.8 mL/min/1.73 m^2 (Grade 4). At the next sample on Day 189, the patient's creatinine value and eGFR had returned to baseline. The event was not considered an AE.

Patient [redacted] (b) (6), a 37-year-old white male, had a low creatinine of 53 µmol/L and normal eGFR of 151.7 mL/min/1.73 m^2 at baseline. On Day 189, the patient had a creatinine of 115 µmol/L (2 x baseline). On Day 357, the patient had an elevated creatinine of 424 µmol/L (Grade 3) and eGFR of 13.7 mL/min/1.73 m^2 (Grade 4). At the next sample on Day 462, the patient's creatinine value and eGFR had returned to baseline. The event was not considered an AE.

Patient [redacted] (b) (6), a 36-year-old white male, had a low creatinine of 53 µmol/L and normal eGFR of 152.6 mL/min/1.73 m^2 at baseline. On Day 189, the patient had an elevated creatinine of 407 µmol/L (Grade 3) and eGFR of 14.5 mL/min/1.73 m^2 (Grade 4). At the next sample on Day 357, the patient's creatinine value and eGFR had returned to baseline. The event was not considered an AE.

Patient [redacted] (b) (6), a 49-year-old white male, had an increased creatinine of 212 (Grade 2) and 168 (Grade 1) µmol/L and decreased eGFR of 28.9 (Grade 3) and 37.9 mL/min/1.73 m^2 (Grade 2) at screening and baseline. On Day 189, the patient had an elevated creatinine of 407 µmol/L (Grade 3) and eGFR of 13.6 mL/min/1.73 m^2 (Grade 4). At the next sample on Day 357, the patient's creatinine value and eGFR had returned to their screening/baseline values with creatinine ranging from 168 to 318 µmol/L and eGFRs ranging from 18.1 to 37.5 mL/min/1.73 m^2. The event was not considered an AE.

Patient [redacted] (b) (6), a 58-year-old white female, had a creatinine of 97 µmol/L and decreased eGFR of 51.2 mL/min/1.73 m^2 (Grade 2) at baseline. On Day 189 and Day 357, the patient had an elevated creatinine of 309 and 734 µmol/L (Grade 3) and eGFR of 13.4 and 4.9 mL/min/1.73 m^2(Grade 4). At the next sample on Day 462, the patient's creatinine value and eGFR had returned to their baseline values. The event was

reported as an AE of blood creatinine increase on Day 205. It was moderate in severity, considered not related and resolved.

<u>Patisiran-LNP patients with creatinine value of >3 × baseline or >4 mg/dL and a concurrent eGFR of 15-29 mL/min/1.73 m ^{2}</u>

Patient [redacted] (b) (6), a 56-year-old white female, had a low creatinine of 44 µmol/L and normal eGFR of 128.3 mL/min/1.73 m ^{2} at baseline. On Day 357 and Day 462, the patient had an elevated creatinine of 168 and 133 µmol/L (Grade 3) and eGFR of 27.2 and 35.7 mL/min/1.73 m ^{2} (Grade 3). At the next sample on Day 546, the patient's creatinine value and eGFR had returned to baseline. The event was not considered an AE.

Patient [redacted] (b) (6), a 41-year-old white female, had a low creatinine of 44 µmol/L and normal eGFR of 136.7 mL/min/1.73 m ^{2} at baseline. On Day 462, the patient had an elevated creatinine of 248 µmol/L (Grade 3) and eGFR of 18.5 mL/min/1.73 m ^{2} (Grade 3). At the next sample on Day 546, the patient's creatinine value and eGFR had returned to baseline. The event was not considered an AE.

<u>Patisiran-LNP patients with eGFR of 15-29 mL/min/1.73 m ^{2}</u>

Patient [redacted] (b) (6), a 62-year-old white male, had an elevated creatinine of 150 µmol/L (Grade 1) and decreased eGFR of 41.1 mL/min/1.73 m ^{2} (Grade 2) at baseline. On Day 189, the patient had an elevated creatinine of 292 µmol/L (Grade 2) and eGFR of 19.1 mL/min/1.73 m ^{2} (Grade 3). At the next sample on Day 357, the patient's creatinine value and eGFR had returned to baseline and stayed stable throughout the study. The event was not considered an AE.

Patient [redacted] (b) (6), a 69-year-old white male with history of moderate renal impairment and congestive heart failure, had a creatinine of 159 (Grade 1) and 124 µmol/L and eGFR of 37.6 and 50.1 mL/min/1.73 m ^{2} (Grade 2) at screening and baseline. On Day 546, the patient had a creatinine of 203 µmol/L (Grade 2) and eGFR of 28.3 mL/min/1.73 m ^{2} (Grade 3). The event was not considered an AE.

Patient [redacted] (b) (6), a 66-year-old Asian male with history of congestive heart failure, diabetes mellitus, atrial flutter, atrioventricular block and hypotension, had a creatinine of 88 µmol/L and eGFR of 75.2 mL/min/1.73 m ^{2} (Grade 1) at baseline. On Day 170, the patient had a SAE of exacerbation of congestive heart failure. On Day 189, the patient had an elevated creatinine of 212 µmol/L (Grade 2) and eGFR of 27.2 mL/min/1.73 m ^{2} (Grade 3). On Day 191, the patient had nonserious AEs reported of blood creatinine increased, BUN increased, and creatinine renal clearance decreased, all moderate,

unlikely or not related to study drug. After Day 191, the patient continued to have worsening of his health status with an SAE of severe diarrhea, and AEs of acute kidney injury, macrocytic anemia, bradycardia, worsening congestive heart failure, hypotension, vomiting, and mild circulatory shock on Day 193. On Day 194, the patient had a cardiac arrest and died. The death was considered not related to study drug.

<u>Placebo group</u>

<u>Placebo patient with creatinine value of >3 × baseline or >4 mg/dL and a concurrent eGFR of 15-29 mL/min/1.73 m²</u>

Patient [redacted] (b) (6), a 45-year-old white female, had a low creatinine of 53 µmol/L and normal eGFR of 108.2 mL/min/1.73 m^2 at baseline. On Day 189, the patient had an elevated creatinine of 186 µmol/L (Grade 3) and eGFR of 25.4 mL/min/1.73 m^2 (Grade 3). At the next sample on Day 357, the patient's creatinine value returned to normal (71 µmol/L) and eGFR was 76.9 mL/min/1.73 m^2. Creatinine and eGFR remained stable for the rest of the study. The event was not considered an AE.

<u>Placebo patients with eGFR of 15-29 mL/min/1.73 m²</u>

Patient [redacted] (b) (6), a 75-year-old white male, with history of renal impairment, hypertension, atrioventricular block, orthostatic hypotension, recurrent urinary tract infections and benign prostatic hypertrophy, had an elevated creatinine of 177 µmol/L (Grade 1) and decreased eGFR of 32.7 mL/min/1.73 m^2 (Grade 2) at baseline. Throughout the study, the patient had creatinine values that ranged from 133 to 168 µmol/L (Grade 1) and eGFRs that ranged from 34.6 to 45.5 mL/min/1.73 m^2. On Day 546, the patient had an elevated creatinine of 212 µmol/L (Grade 2) and eGFR of 26.5 mL/min/1.73 m^2. The patient had an AE of worsening renal function on Day 150-Day 161.

Patient [redacted] (b) (6), a 52-year-old white female, with history of renal impairment, atrioventricular block, pacemaker placement and nephrogenic anemia, had an elevated creatinine of 150 µmol/L (Grade 1) and decreased eGFR of 31.6 mL/min/1.73 m^2 (Grade 2) at baseline. Throughout the study, the patient had creatinine values that ranged from 141 to 177 µmol/L (Grade 1) and eGFRs that ranged from 26.0 to 33.8 mL/min/1.73 m^2. During the study, the patient had an SAE of dehydration on Day 141, had an SAE of diarrhea, acute kidney injury and urinary tract infection on Day 181, and an SAE of dehydration and hypokalemia on Day 273. The patient withdrew from the study on Day 470 due to posterior reversible encephalopathy syndrome. On Day 470, the patient had an elevated creatinine of 141 µmol/L (Grade 1) and eGFR of 33.7 mL/min/1.73 m^2(Grade 2).

13.11. Premedication-related Adverse Events in Study 004. Source: Study 004 CSR, p. 1028

System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
At Least 1 AE	28 (36.4)/105	54 (36.5)/160
Ear and labyrinth disorders	0	1 (0.7)/1
Deafness unilateral	0	1 (0.7)/1
Endocrine disorders	1 (1.3)/1	1 (0.7)/1
Cushingoid	1 (1.3)/1	1 (0.7)/1
Eye disorders	0	2 (1.4)/3
Cataract	0	1 (0.7)/2
Glaucoma	0	1 (0.7)/1
Gastrointestinal disorders	5 (6.5)/7	6 (4.1)/12
Abdominal discomfort	1 (1.3)/1	0
Abdominal distension	1 (1.3)/1	0
Abdominal pain upper	1 (1.3)/1	0
Diarrhoea	1 (1.3)/1	1 (0.7)/2
Dry mouth	0	1 (0.7)/1
Dyspepsia	0	2 (1.4)/6
Gastritis	1 (1.3)/1	1 (0.7)/1
Gastrooesophageal reflux disease	0	1 (0.7)/1
Hypoaesthesia oral	1 (1.3)/1	0
Mouth ulceration	0	1 (0.7)/1
Oesophagitis	1 (1.3)/1	0
General disorders and administration site conditions	4 (5.2)/4	15 (10.1)/39
Asthenia	2 (2.6)/2	2 (1.4)/9
Chest discomfort	1 (1.3)/1	0
Drug intolerance	0	4 (2.7)/4
Feeling hot	0	2 (1.4)/3
Idiosyncratic drug reaction	0	1 (0.7)/1
Infusion site extravasation	0	1 (0.7)/1
Infusion site paraesthesia	0	1 (0.7)/1
Injection site discomfort	0	1 (0.7)/3
Injection site erythema	0	1 (0.7)/6
Injection site pain	0	1 (0.7)/1
Injection site paraesthesia	0	1 (0.7)/3
Oedema	0	1 (0.7)/1
Oedema peripheral	1 (1.3)/1	3 (2.0)/6
Immune system disorders	0	1 (0.7)/1
Infusion related reaction	0	1 (0.7)/1
Infections and infestations	3 (3.9)/3	7 (4.7)/8

System Organ Class/ Preferred Term	Number of patients (%) [1]/Events [2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Erysipelas	1 (1.3)/1	2 (1.4)/2
Folliculitis	0	1 (0.7)/1
Helicobacter gastritis	0	1 (0.7)/1
Oral herpes	0	1 (0.7)/1
Respiratory tract infection	1 (1.3)/1	0
Urinary tract infection	1 (1.3)/1	1 (0.7)/2
Viral rash	0	1 (0.7)/1
Injury, poisoning and procedural complications	2 (2.6)/2	2 (1.4)/2
Fall	0	1 (0.7)/1
Foot fracture	1 (1.3)/1	0
Spinal compression fracture	1 (1.3)/1	1 (0.7)/1
Investigations	0	1 (0.7)/1
Weight increased	0	1 (0.7)/1
Metabolism and nutrition disorders	1 (1.3)/2	4 (2.7)/4
Glucose tolerance impaired	0	1 (0.7)/1
Hyperglycaemia	1 (1.3)/2	1 (0.7)/1
Vitamin D deficiency	0	2 (1.4)/2
Musculoskeletal and connective tissue disorders	3 (3.9)/3	5 (3.4)/5
Musculoskeletal pain	0	1 (0.7)/1
Osteopenia	0	1 (0.7)/1
Osteoporosis	3 (3.9)/3	3 (2.0)/3
Nervous system disorders	8 (10.4)/48	13 (8.8)/25
Burning sensation	0	1 (0.7)/2
Dizziness	3 (3.9)/28	4 (2.7)/4
Headache	1 (1.3)/1	0
Hypoaesthesia	1 (1.3)/1	0
Paraesthesia	0	3 (2.0)/10
Psychomotor hyperactivity	1 (1.3)/12	0
Restless legs syndrome	1 (1.3)/1	3 (2.0)/3
Somnolence	3 (3.9)/3	2 (1.4)/4
Tremor	1 (1.3)/2	0
Visual field defect	0	2 (1.4)/2
Psychiatric disorders	6 (7.8)/12	14 (9.5)/22
Anxiety	0	1 (0.7)/1
Depression	1 (1.3)/1	0
Insomnia	3 (3.9)/8	10 (6.8)/18
Irritability	2 (2.6)/2	0
Restlessness	1 (1.3)/1	1 (0.7)/1

System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Sleep disorder	0	1 (0.7)/1
Tachyphrenia	0	1 (0.7)/1
Renal and urinary disorders	1 (1.3)/1	1 (0.7)/3
Glycosuria	0	1 (0.7)/3
Urinary retention	1 (1.3)/1	0
Reproductive system and breast disorders	0	1 (0.7)/1
Menstruation irregular	0	1 (0.7)/1
Respiratory, thoracic and mediastinal disorders	1 (1.3)/1	2 (1.4)/6
Dyspnoea	0	1 (0.7)/3
Hiccups	0	1 (0.7)/3
Pulmonary oedema	1 (1.3)/1	0
Skin and subcutaneous tissue disorders	6 (7.8)/6	8 (5.4)/13
Acne	0	1 (0.7)/1
Cold sweat	1 (1.3)/1	0
Decubitus ulcer	1 (1.3)/1	0
Eczema	0	1 (0.7)/1
Erythema	0	3 (2.0)/5
Hair growth abnormal	0	1 (0.7)/1

System Organ Class/ Preferred Term	Number of patients (%)[1]/Events[2] Placebo (N=77)	Patisiran 0.3 mg/kg (N=148)
Night sweats	1 (1.3)/1	2 (1.4)/3
Purpura	1 (1.3)/1	0
Rash	1 (1.3)/1	0
Skin fragility	0	1 (0.7)/1
Skin ulcer	1 (1.3)/1	0
Swelling face	0	1 (0.7)/1
Vascular disorders	2 (2.6)/15	4 (2.7)/13
Flushing	2 (2.6)/15	2 (1.4)/11
Hot flush	0	1 (0.7)/1
Hypertension	0	1 (0.7)/1

13.12. Protocol Amendments for Study 004

Version Number	Date	Summary of Changes
1.0 – Global Original	15 Aug 2013	NA
1.1 - France	06 Dec 2013	In response to EC request, added Exclusion Criterion #24 "patients under legal protection"
2.0 – Global Amendment 1.0	18 Oct 2013	• Reordered secondary endpoints and modified methods of analysis • Modified Inclusion Criterion #7 to allow an INR of ≤3 only for patients on warfarin • Clarified definition of "highly effective birth control" • Modified the premedication regimen to be administered the night prior to study drug administration and on the day of study drug administration
2.1 – Italy-specific Amendment 1.1	23 Jan 2014	• Added the following country-specific inclusion criterion: Must not, in the principal Investigator's opinion, be a candidate for tafamidis
2.1 – Portugal-specific	06 Jan 2014	

Version Number	Date	Summary of Changes
Amendment 1.1		
2.1 –Taiwan-specific Amendment 1.1	13 Feb 2014	• Increased minimum inclusion age from 18 to 20 years old
2.1 – France-specific Amendment 1.1	14 Jan 2014	• Modified Inclusion Criterion #6: platelet count changed from ≥100,000 cells/mm³ to ≥50000 cells/mm³
3.0 –Global Amendment 2.0	21 Mar 2014	• Clarified that entry criteria, besides Inclusion Criteria #3 and #4, would continue to be assessed at both the Screening and the Screening/Baseline visits • Modified Inclusion Criterion #1 to allow for enrollment of subjects up to 85 years of age (inclusive) • Modified Inclusion Criterion #3: the lower limit of the NIS changed from 10 to 5 • Modified Inclusion Criterion #4 to include patients with a NCS sum of the sural sensory nerve action potential, the tibial compound muscle action potential and the peroneal CMAP ≥2 • Modified Inclusion Criterion #6: platelet count changed from ≥100,000 cells/mm³ to ≥50000 cells/mm³ • Modified Inclusion Criterion #7: INR value changed from ≤3 to ≤3.5 • Clarified Exclusion Criterion #1 to exclude patients with vitamin A levels consistent with vitamin A deficiency • Removed Exclusion Criterion #18 (removed: Participated in a clinical study with an antisense oligonucleotide for more than 3 months; if in a clinical study with antisense oligonucleotide for ≤3 months, must have completed a 3-month wash-out prior to start of study drug administration in this study) • Removed Diflunisal from Exclusion Criterion #19 and added to Exclusion Criterion #20 to clarify that a 3-day washout period prior to start of study drug for this particular agent
4.0 – Global Amendment 3.0	24 Apr 2014	• Expanded the screening window from 28 days to 42 days to accommodate traveling patients
4.1 –Italy-specific Amendment 3.1	01 May 2014	• Incorporated Global Amendment 2 and 3 changes
4.1 – Portugal-specific Amendment 3.1	01 May 2014	
4.1 –Taiwan-specific Amendment 3.1	01 May 2014	
4.1 – France-specific Amendment 3.1	02 Jul 2014	
4.1 – Netherlands specific amendment	16 Sept 2014	• In response to EC requests, Exclusion Criterion #2 was modified to remove "or is planning to undergo liver transplant during the study period" • Added study schematic.

Version Number	Date	Summary of Changes
5.0 – Global Amendment 4.0	04 Aug 2014	• Modified Inclusion Criterion #3: the upper limit NIS changed from 100 to 130, and the requirement for having a PND score of ≤3b was added • Modified Inclusion Criterion #7 to remove albumin criterion and to increase INR criterion from ≤1.2 to ≤2.0 • Modified Inclusion Criterion #8: serum creatinine changed from ≤1.5 to ≤2 × ULN • Modified Inclusion Criterion #9 to exclude only patients with an active hepatitis B or hepatitis C infection • Modified Inclusion Criterion #1 to change period from 1 month to 75 days after last dose of study drug for women of child-bearing potential • Modified Inclusion Criterion #11 to extend the period that males with partners of child-bearing potential must use 1 barrier method and 1 additional method of contraception from 1 month to 75 days after the last dose of study drug • Removed Exclusion Criterion #1 (has vitamin A level consistent with vitamin A deficiency) • Clarified Exclusion Criterion #16 to state that patients with a history of alcohol abuse within the past 2 years or daily heavy alcohol consumption • Modified Exclusion Criterion #17 to exclude patients who participated in a clinical study with antisense oligonucleotide unless there is a 3- month wash-out period • Modified Exclusion Criterion #24 to define "under legal protection"
5.1 – Italy-specific Amendment 4.1	04 Aug 2014	• Incorporated Global Amendment 4 changes
5.1 – Portugal-specific Amendment 4.1	04 Aug 2014	
5.1 – Taiwan-specific Amendment 4.1	04 Aug 2014	
5.1 – France-specific Amendment 4.1	04 Aug 2014	• Clarified that patients with mental incapacity were not suitable for enrollment • Incorporated Global Amendment 4 changes
5.1 – Netherlands-specific Amendment 4.1	06 Nov 2014	• Permitted patients to stay on the liver transplant list while participating in the study • Incorporated Global Amendment 4 changes
5.1 – Japan-specific Amendment 4.1	27 Aug 2014	• Added specific requirements for menopausal women
5.1 – Brazil-specific Amendment 4.1	09 Mar 2015	• Added serum or urine pregnancy testing before each dose of patisiran-LNP
6.0 – Global	08 Sep 2015	• Implemented a reduced dose of dexamethasone for the protocol-

Version Number	Date	Summary of Changes
Amendment 5.0		specified premedication regimen on the day of study drug administration, and removed administration of premedication the night before study drug administration. • Specified that patients who are intolerant of 110 mg IV dexamethasone or equivalent on the day of infusion may be considered for further stepwise reduction in dexamethasone or equivalent after consultation with the Medical Monitor • Updated the risk benefit assessment to reflect liver function test abnormalities and risk for osteoporosis • Modified Inclusion Criterion #4: added ulnar SNAP and ulnar CMAP measurements to the qualifying NCS • Modified Inclusion Criterion #7: permitted enrollment of patients with a total bilirubin level elevation to ≤2 x upper limit of normal • Modified Exclusion Criterion #14: clarified that patients with any uncontrolled cardiac arrhythmia or unstable angina are not permitted to enroll in the study • Included the option for patients to permanently discontinue study treatment and remain on study
6.1 – Italy-specific Amendment 5.1	09 Sep 2015	• Incorporated Global Amendment 5 changes
6.1 – Portugal-specific Amendment 5.1	09 Sep 2015	
6.1 – Taiwan-specific Amendment 5.1	09 Sep 2015	
6.1 – France-specific Amendment 5.1	09 Sep 2015	
6.1 – Netherlands-specific Amendment 5.1	09 Sep 2015	
6.1 – Japan-specific Amendment 5.1	09 Sep 2015	
6.1 – Brazil-specific Amendment 5.1	23 Sep 2015	
6.1 – Germany-specific Amendment 5.1	17 Dec 2015	• Addition of MR neurography as an exploratory objective with assessments in 6-month intervals
6.2 – France-specific Amendment 5.2	17 Dec 2015	
Global Administrative letter	04 Jan 2017	• Added assessment of dermal amyloid burden using the same skin punch biopsies collected for IENFD and SGNFD assessments

Version Number	Date	Summary of Changes

Abbreviations: CMAP= Compound muscle action potential; EC=ethics committee; IENFD=intraepidermal nerve fiber density; INR= international normalized ratio; MR=magnetic resonance; NCS=nerve conduction study; NIS=Neurologic Impairment Score; PND=polyneuropathy score; SGNFD=sweat gland nerve fiber density; SNAP= sensory nerve action potential

13.13. ECG Shift Plots for Study 004

Figure 17: Bivariate Fit of QTCB Trial Mean By QTCB Baseline: QTCB = corrected QT interval using Bazett's formula, in milliseconds

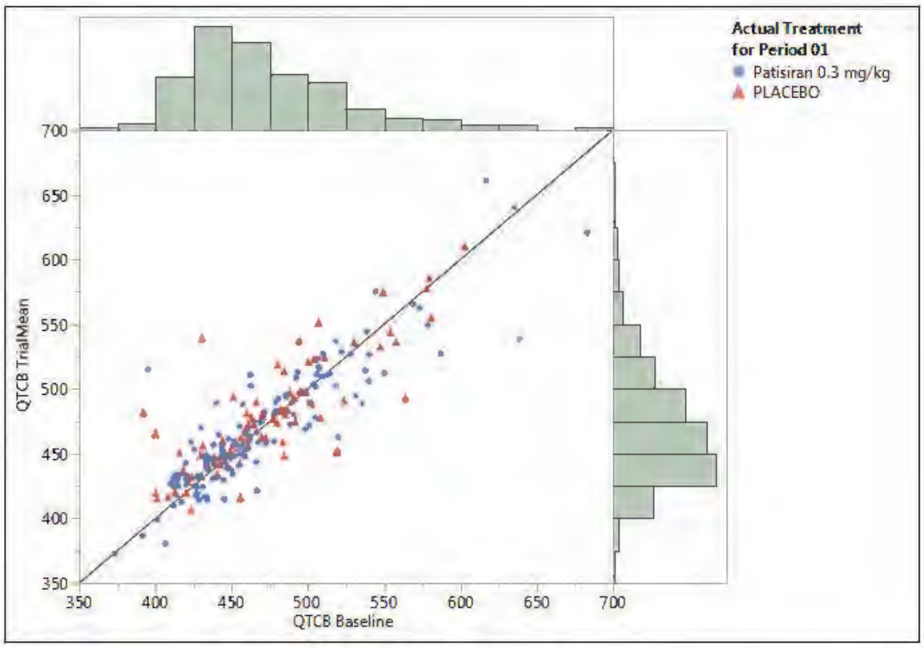


Figure 18: Bivariate Fit of QTCF Trial Mean By QTCF Baseline: QTCF = QT interval using Fridericia's Correction Formula, in milliseconds

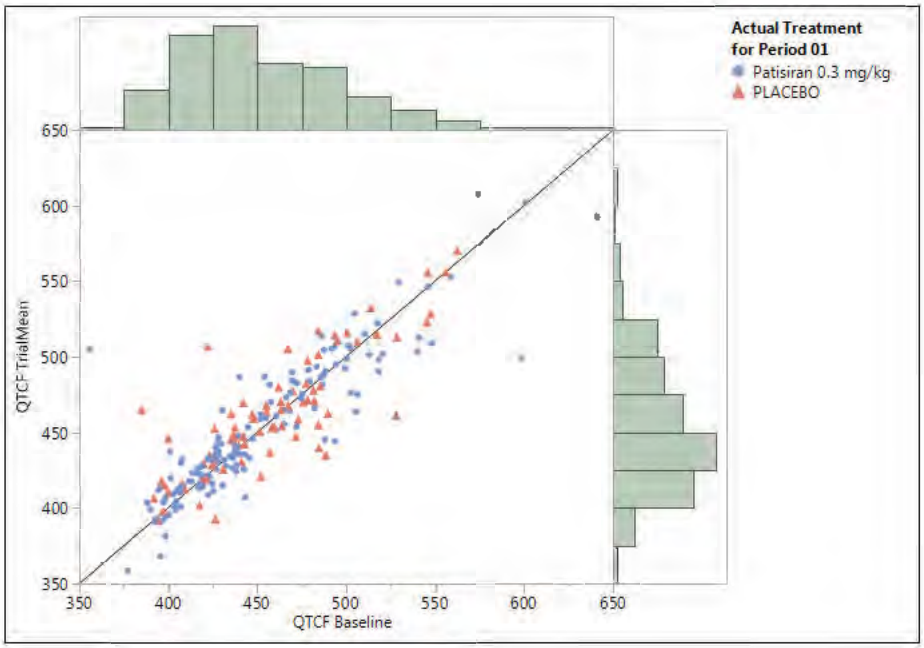


Figure 19: Bivariate Fit of PRMEAN Trial Mean By PRMEAN Baseline: PRMEAN = Mean PR interval in milliseconds.

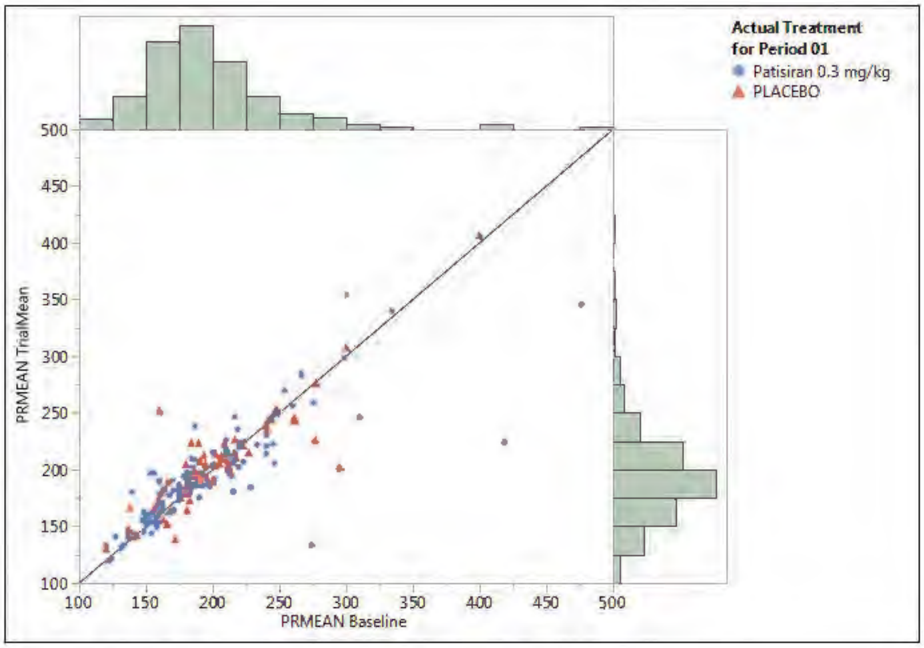
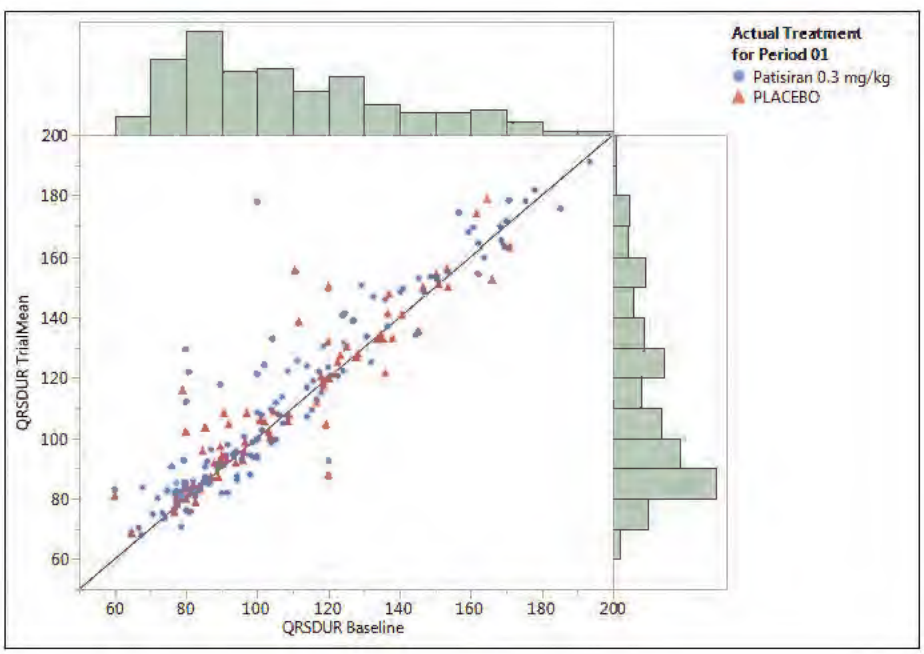


Figure 20: Bivariate Fit of QRSDUR Trial Mean By QRSDUR Baseline: QRSDUR = QRS interval in milliseconds.



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/s/

NICHOLAS A KOZAUER on behalf of RAINER PAINE
08/06/2018

NICHOLAS A KOZAUER
08/06/2018

FDA U.S. FOOD & DRUG ADMINISTRATION

Date: July 27, 2018
From: Kimberly Smith, Medical Officer, Division of Cardiovascular and Renal Products
Through: Aliza Thompson, Team Leader
Norman Stockbridge, Director
Division of Cardiovascular and Renal Products
To: Nick Kozauer, CDTL, Division of Neurology Products
Subject: Renal safety of patisiran – follow-up

Background
Patisiran is a small interfering RNA (siRNA) molecule developed for the treatment of hereditary transthyretin (hATTR) amyloidosis with polyneuropathy, a condition caused by mutations in the transthyretin (TTR) gene that lead to the accumulation of amyloid fibrils and plaques in multiple tissues. On December 11, 2017, the Division of Neurology Products (DNP) received an NDA for patisiran for the treatment of adults with hATTR amyloidosis based on the results of study ALN-TTR02-004. DNP requested that the Division of Cardiovascular and Renal Products review five cases of decreases in renal function. DCRP filed an initial response to the consult on July 12, 2018 and recommended that the applicant seek additional details regarding these cases. This addendum reviews the applicant's response, which was submitted July 18, 2018.

Summary of New Information
The applicant obtained additional information from two clinical sites: Site 060 in Spain and Site 110 in Mexico. The investigator for Site 110 noted "patients had multiple causes that explained the changes in renal function and because the laboratory samples were repeated locally and showed improvement, the investigators of the site did not attribute these changes to the drug but to the natural history of the disease in advance stages." Of note, all five subjects remained on study drug until the planned end of treatment.

Relevant new information is as follows (see our consult dated July 12, 2018 for additional details):

Subject (b) (6): Baseline serum creatinine was 0.6 mg/dL. On Day 83, the serum creatinine was 4.7 mg/dL and eGFR was 13 mL/min/1.73 m^2. According to the site, the sample was hemolyzed with an abnormal potassium (6.9 mmol/L) and the elevated creatinine was attributed to the hemolyzed sample. The patient "had no alarming symptoms" and no further workup was performed. No local labs were performed. On Day 190, the serum creatinine was 0.6 mg/dL.

Reviewer's comment: While hemolysis of blood samples can increase some serum chemistry values, we would not expect it to increase serum creatinine to the degree observed in this subject. In addition, severe AKI can be asymptomatic.

Subject (b) (6): The subject had NYHA Class II heart failure, orthostatic hypotension (fall in systolic blood pressure of >30 mmHg with standing) requiring fludrocortisone, and severe diarrhea requiring loperamide. On Day 192, serum creatinine increased from a baseline of 1.9 mg/dL to 4.6 mg/dL. The patient started oral rehydration therapy. A local measurement performed on Day 216 showed a creatinine of 1.8 mg/dL. Creatinine fluctuated between 1.9 and 3.6 mg/dL during the trial. Adverse events reported during this time included six events of moderate to severe diarrhea with each event lasting up to 2 weeks, a urinary tract infection treated with ciprofloxacin, and peripheral edema treated with furosemide. The fluctuations in renal function were attributed to these events.

Reviewer's comment: The subject had baseline renal insufficiency, NYHA Class II heart failure, orthostatic hypotension, recurrent diarrhea, a urinary tract infection, and diuretic use during the trial. Although the report is lacking in some details such as whether the reported acute events were temporally related to declines in renal function, it seems likely that the fluctuations in renal function were related to these issues.

Subject [redacted] (b) (6)*:* Baseline serum creatinine was 1.1 mg/dL. On Day 125, the subject had a urinary tract infection that was treated with fosfomycin. On Day 191, serum creatinine was 3.5 mg/d, which was reported as an AE on Day 205. On Day 207, serum creatinine was 0.7 mg/dL on local labs. She was diagnosed with a urinary tract infection the same day and again on Day 259 and was treated with ciprofloxacin both times. On Day 359, serum creatinine was 8.3 mg/dL. On Day 373, serum creatinine was 0.6 mg/dL on local labs, and the patient was asymptomatic, so no further evaluation was performed. "According to the PI, the cause of the transient worsening of renal function was attributed to recurrent or refractory urinary tract infections."

Reviewer's comments: The narrative does not have sufficient detail to understand what transpired, but it is possible that the urinary tract infections were temporally related to the declines in renal function and were severe enough to result in AKI.

Subject [redacted] (b) (6)*:* The subject had a history of diarrhea requiring loperamide and, during the study, had multiple episodes of moderate to severe diarrhea lasting up to two weeks and treated with loperamide. The subject also had orthostatic hypotension with a fall in systolic blood pressure of >30 mmHg with standing during study testing. Baseline serum creatinine was 0.6 mg/dL. On Day 358, serum creatinine was 4.8 mg/dL. On Day 409, serum creatinine was 0.7 mg/dL on local labs, so no further work-up was performed. "According to the PI, the transient worsening of renal function was attributed to renal hypoperfusion due to hemodynamic changes secondary to recurrent bouts of moderate to severe diarrhea and dehydration. This was further supported by the severe orthostatic hypotension demonstrated through the study."

Reviewer's comment: In previous submissions, the applicant noted that episodes of worsening diarrhea were reported on Days 336-341 and 359-364, around the time serum creatinine was elevated on Day 358.

Subject [redacted] (b) (6)*:* The subject had a history of diarrhea requiring loperamide and had orthostatic hypotension with a fall in systolic blood pressure of >30 mmHg with standing during study testing. According to the applicant, the patient had several adverse events of diarrhea that were moderate or severe in intensity (timing not provided) before the decline in eGFR. Baseline serum creatinine was 0.6 mg/dL. On Day 189, serum creatinine was 4.6 mg/dL. On Day 203, serum creatinine was 1.0 mg/dL on local labs. The patient was asymptomatic, so no further work-up was performed.

Reviewer's comment: It is not clear whether the episodes of diarrhea were temporally related to the decline in renal function, but it is possible that volume depletion related to diarrhea was the underlying etiology.

DCRP Conclusion

Based on the additional information provided, some of the cases of severe AKI may have been associated with hemodynamic insults (e.g., volume depletion from diarrhea). Because significant declines in renal function do not appear to have been recognized or evaluated in a timely fashion, there are still significant gaps in our understanding of the nature of these events.

It is our understanding from the primary review team that preclinical data and analyses of other trial data do not suggest that patisiran is associated with renal toxicity. As such, we defer to the primary review team regarding what, if anything, should be said about these cases in the label.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

KIMBERLY A SMITH
07/27/2018

ALIZA M THOMPSON
07/27/2018

NORMAN L STOCKBRIDGE
07/27/2018

U.S. FOOD & DRUG ADMINISTRATION

Date: July 11, 2018
From: Kimberly Smith, Medical Officer, Division of Cardiovascular and Renal Products
Through: Aliza Thompson, Team Leader
Norman Stockbridge, Director
Division of Cardiovascular and Renal Products
To: Nick Kozauer, CDTL, Division of Neurology Products
Subject: Renal safety of patisiran

Background

Patisiran is a small interfering RNA (siRNA) molecule developed for the treatment of hereditary transthyretin (hATTR) amyloidosis with polyneuropathy, a condition caused by mutations in the transthyretin (TTR) gene that lead to the accumulation of amyloid fibrils and plaques in multiple tissues. Clinical manifestations of hATTR amyloidosis vary but generally include neuropathy, cardiomyopathy, or both. Renal involvement is rare. Patisiran targets a conserved region in the 3' untranslated region of wild type and mutant TTR mRNA and, through RNA interference, leads to degradation of TTR mRNA in the liver, a reduction of serum TTR protein, and a reduction in amyloid deposits in tissues. Patisiran is formulated as lipid nanoparticles to facilitate delivery to hepatocytes where that majority of TTR is produced. Of note, patisiran did not cause renal toxicity in preclinical studies.

On December 11, 2017, the Division of Neurology Products (DNP) received an NDA for patisiran for the treatment of adults with hATTR amyloidosis based on the results of study ALN-TTR02-004. DNP has requested review by the Division of Cardiovascular and Renal Products of "five cases of significant but transient abnormalities in renal function on treatment" in this study (Subjects [redacted] (b) (6) [redacted]) and comment on whether any description is warranted in labeling and if additional postmarketing assessment is necessary.

Materials Reviewed

1. Summary of Clinical Safety
2. Response to July 6, 2018 clinical information request submitted July 9, 2018
3. 16.2.8 Listing of Individual Laboratory Measurements by Patient
4. 16.2.1.1 Patient Disposition
5. Nonclinical Overview

Overview of Protocol

ALN-TTR02-004 was a randomized, double-blind, placebo-controlled study in 225 patients with hATTR amyloidosis with a TTR mutation and symptomatic polyneuropathy. Patients were randomized 2:1 to receive intravenous patisiran 0.3 mg/kg or placebo every 3 weeks for 18 months. The primary objective was to determine the efficacy of patisiran by evaluating the difference between the patisiran and placebo groups in the change from baseline of Modified Neuropathy Impairment Score (mNIS+7) at 18 months.

Patients were required to have a serum creatinine ≤ 2x ULN at baseline. Serum creatinine was assessed at screening and on Days 0, 84, 189, 357, 462, 546, and 602 (follow-up). A urinalysis was performed at screening and on Days 252 and 546.

Summary of Cases
The applicant identified five subjects in the patisiran group with a creatinine value of >3 x baseline or >4 mg/dL and a concurrent eGFR <15 mL/min/1.73m^2 compared with none in the placebo group. Summaries of the information provided in the Summary of Clinical Safety and in response to an information request asking for additional details regarding these cases are as follows:

Subject [redacted] (b) (6) was a 60-year-old white male with a history of neuropathy, NYHA Class I heart failure, atrial fibrillation, and normal baseline renal function with a serum creatinine of 0.6 mg/dL and eGFR of 138 mL/min/1.73 m^2. At the first post-baseline assessment on Day 84, his creatinine was 4.6 mg/dL and eGFR was 13 mL/min/1.73 m^2. His BUN and potassium were elevated at 43 mg/dL and 6.9 mmol/L, respectively, in the same sample. At the next assessment on Day 189, his renal function had returned to baseline (eGFR 137 mL/min/1.73 m^2), and it remained at baseline through Day 567. A urinalysis was assessed at screening and on Day 545; neither showed significant abnormalities. The event was not reported as an AE, and no other AEs were reported around the time of the event. No evaluation was performed at the time of the low eGFR. No changes in study drug, concomitant medications, or other interventions were reported. The subject completed 27 doses (4 prior to the eGFR reduction) without interruption.

Subject [redacted] (b) (6) was a 49-year-old white male with a history of neuropathy, NYHA Class II heart failure, orthostatic hypotension, diarrhea, and reduced renal function with screening and baseline serum creatinine values of 2.4 and 1.9 mg/dL and eGFR values of 29 and 38 mL/min/1.73 m^2, respectively. On Day 189, his creatinine was 4.6 mg/dL and eGFR was 14 mL/min/1.73 m^2. The BUN was mildly elevated at 26 mg/dL. At the next sample on Day 357, his renal function had returned to baseline (serum creatinine 2.2 mg/dL, eGFR 32 mL/min/1.73 m^2). His eGFR fell once again to 18 mL/min/1.73 m^2 by the next sample on Day 462 but then returned to baseline (eGFR of 32 to 38 mL/min/1.73 m^2) on three samples drawn between Days 546 and 567. A urinalysis was assessed at screening and on Days 252 and 546; none showed significant abnormalities. The events were not reported as AEs, and no other AEs were reported around the same time. No evaluation was performed at the time of the low eGFR. No changes in study drug, concomitant medications, or other interventions were reported. The subject completed 27 doses (9 prior to the eGFR first reduction) without interruption.

Subject [redacted] (b) (6) was a 58-year-old white female with a history of neuropathy, NYHA Class II heart failure, and reduced renal function with a baseline serum creatinine of 1.1 mg/dL and eGFR of 51 mL/min/1.73 m^2. On Day 84, her renal function remained at baseline. On Days 189 and Day 357, her serum creatinine was 3.5 and 8.3 mg/dL, respectively, and her eGFR was 13 and 5 mL/min/1.73 m^2, respectively. BUN was in the normal range at both time points. At the next sample on Day 462, her creatinine and eGFR had returned to their baseline values and remained there through Day 567. On Day 205, the event was reported as an AE of blood creatinine increase of moderate in severity that was not related to study drug and was later reported as resolved. A urinalysis was assessed at screening and on Days 252 and 546; none showed significant abnormalities. The subject experienced repeated "urinary tract infections throughout the study" and was treated with fosfomycin (starting on Day 125) and ciprofloxacin (starting on Days 207, 260, 422, and 545). No adverse events were reported around the times of the low eGFR. No evaluation was performed at the time of the low eGFR. No changes in study drug, concomitant medications, or other interventions were reported related to the decline in eGFR. The subject completed 27 doses (9 prior to the first eGFR reduction and 17 doses prior to the second) without interruption.

Reviewer comment: The applicant notes that subject [redacted] (b) (6) *had repeated urinary tract infections during the study treated with antibiotics. The basis for the diagnosis of urinary tract infections was not provided, and it is not obvious that the urinary tract infections or antibiotics contributed to the decline in renal function.*

Subject [redacted] (b) (6) was a 37-year-old white male with history of neuropathy, NYHA Class II heart failure, diarrhea, and normal renal function with a baseline serum creatinine of 0.6 mg/dL and eGFR of 152 mL/min/1.73 m^2. On Day 84, his renal function remained at baseline. At the next assessment on Day 189, his creatinine was 1.3 mg/dL and eGFR was 62 mL/min/1.73 m^2. On Day 357, his creatinine was 4.8 mg/dL and eGFR was 14 mL/min/1.73 m^2. BUN was in the normal range. By the next sample on Day 462, his renal function had returned to baseline and it remained at baseline through Day 567. A urinalysis was assessed at screening and on Days 252 and 546; none showed significant abnormalities. The event was not reported as an AE. The patient had recurrent episodes of worsening of diarrhea during the study with two episodes around the time of the low eGFR (Days 336-341 and 359-364). No evaluation was performed at the time of the low eGFR. No changes in study drug, concomitant medications, or other interventions were reported. The subject completed 27 doses (17 prior to the eGFR reduction) without interruption.

Subject [redacted] (b) (6) was a 36-year-old white male with a history of neuropathy, NYHA Class II heart failure, diarrhea, and normal renal function with a baseline creatinine of 0.6 mg/dL and eGFR of 153 mL/min/1.73 m^2. On Day 84, his serum creatinine and eGFR remained at baseline. At the next assessment on Day 189, his creatinine was 4.6 mg/dL and eGFR was 15 mL/min/1.73 m^2. BUN was in the normal range. By the next sample on Day 357, his renal function had returned to baseline, and it remained at baseline through Day 567. A urinalysis was assessed at screening and on Days 252 and 546; none showed significant abnormalities. The event was not reported as an AE. The patient had recurrent episodes of worsening of diarrhea during the study with two episodes around the time of the low eGFR (Days 171-173 and 191-195). No evaluation was performed at the time of the low eGFR. No changes in study drug, concomitant medications, or other interventions were reported. The subject completed 27 doses (9 prior to the eGFR reduction) without interruption.

Reviewer comment: The applicant notes that subjects [redacted] (b) (6) *and* [redacted] (b) (6) *had worsening of baseline diarrhea around the time renal function was reduced; however, BUN was normal, which is not consistent with a prerenal etiology of acute kidney injury.*

Consult Questions
There were 5 cases of significant but transient abnormalities in renal function on treatment in the placebo-controlled clinical trial in this application (Subjects [redacted] (b) (6)) with no similar cases in placebo. We would greatly appreciate your evaluation of these cases with specific respect to whether any description of this finding is warranted in labeling and if additional postmarketing assessment would be necessary.

DCRP Response: The applicant identified five subjects in the patisiran group who experienced a substantial decline in renal function during the study defined as a creatinine value of >3x baseline or to >4 mg/dL with a concurrent eGFR <15 mL/min/1.73m^2. The narratives submitted for these cases were limited primarily to laboratory parameters measured during routine study visits; therefore, during the course of our review, we requested that the applicant "provide detailed medical narratives for these cases that include, as available, a description of any relevant comorbidities or concomitant medications; clinical events that occurred proximate to the decline in renal function; the results of other renal function assessments from around the time of the event that speak to the time course of the development and resolution of the event; the results of any evaluations performed to determine the etiology of the decline in renal function; and whether the event led to any changes in study drug, concomitant medications, or other interventions."

The applicant responded to our request on July 9, 2019; however, the response did not contain substantial additional details. As such, it is difficult for us to comment on these cases, including whether

they were clinically significant and/or could have been drug-related. According to the applicant, renal function returned to baseline despite continuation of therapy. Although this is somewhat reassuring, we believe additional information is needed to characterize these events and determine whether patisiran may have played a role. We recommend that the applicant obtain the additional details requested in our July 6, 2018 clinical information request through review of site or other medical records. If this issue cannot be resolved prior to the action date, then consideration should be given to including language in labeling related to this finding, although it will be challenging to determine what to communicate given the data we have reviewed to date.

We note that four of the identified subjects were from Site 110. Given that substantial declines in renal function were not reported as adverse events or, based on information provided thus far, otherwise investigated, we think these cases may raise larger questions about the integrity of the data and/or conduct of the study at this site. You may want to consider this issue further.

Additional Comment:
In your consult request, you asked us to review five specific cases of markedly reduced renal function. We note that the applicant's case definition (i.e., creatinine value of >3 x baseline or >4 mg/dL and a concurrent eGFR <15 mL/min/1.73m^2*) is not one that is typically used to evaluate a product's potential to cause nephrotoxicity. We assume that more standard/typical analyses have also been conducted to evaluate the renal safety of patisiran. Please let us know if you need additional guidance on this issue.*

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

KIMBERLY A SMITH
07/11/2018

ALIZA M THOMPSON
07/11/2018

NORMAN L STOCKBRIDGE
07/12/2018

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	210922
Supporting document:	5
Applicant's letter date:	November 15, 2017
CDER stamp date:	November 15, 2017
Product:	Patisiran-LNP
Indication:	Hereditary Transthyretin-Mediated Amyloidosis
Applicant:	Alnylam Pharmaceuticals, Inc. (Alnylam)
Review Division:	Neurology Products
Reviewer:	David L. Carbone, Ph.D.
Supervisor:	Lois M. Freed, Ph.D.
Division Director:	Billy Dunn, M.D.
Project Manager:	Annie Nguyen, R.Ph.

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 210922 are owned by Alnylam or are data for which Alnylam has obtained a written right of reference. Any information or data necessary for approval of NDA 210922 that Alnylam does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application are for descriptive purposes only and are not relied upon for approval of NDA 210922.

TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

ALN-TTR02 (Patisiran-LNP; ONPATTRO) was developed by Alnylam Pharmaceuticals (Alnylam) for the treatment of hereditary transthyretin-mediated amyloidosis. ALN-TTR02 is an siRNA against transthyretin (TTR) mRNA.

1.2 Brief Discussion of Nonclinical Findings

ALN-TTR02 is intended to suppress hepatic TTR production. According to the sponsor, the siRNA sequence in ALN-TTR02 (ALN-18328) is conserved among human WT and known mutant sequences of the TTR gene, as well as being complimentary to the monkey TTR gene. To achieve liver-specific drug delivery, the lipid nanoparticle (LNP) in which the siRNA is encapsulated is thought to interact with apolipoprotein E following IV administration, resulting in subsequent hepatocyte uptake by LDL receptors. There are no nonclinical safety concerns regarding excipients, impurities or degradation products.

Primary pharmacology studies in monkeys administered ALN-TTR02 by IV infusion resulted in decreases in TTR mRNA and circulating TTR protein, with effects lasting up to 28 days. An additional study in transgenic mice that express human mutant TTR indicated significant reductions of TTR protein immunoreactivity in esophagus, colon, stomach, sciatic nerve, and dorsal ganglion following six, twice weekly IV doses of ALN-TTR02. Reversible, off-target effects in monkeys included reductions in circulating retinol binding protein, vitamin A, and T_4 following IV infusion with ALN-TTR02; however, such effects are expected given their respective interactions with TTR. Transient increases in HR were observed in safety pharmacology studies conducted in cynomolgus monkeys; there were no drug effects on CNS or respiratory parameters.

ALN-TTR02 was generally well tolerated in single dose studies in cynomolgus monkeys as well as repeat dose studies of 26 and 39 weeks' duration in male and female SD rats and cynomolgus monkeys, respectively. Primary toxicity included elevations in liver function tests in both species, with correlations to hepatocyte vacuolation (rat and monkey), and single cell necrosis, reactive sinusoids, mixed cell infiltration, and pigment deposition in monkeys. Drug-related toxicity in rats and monkeys generally resolved over 12- and 13-week recovery periods, respectively. The NOAEL in rat and monkey was 0.3 mg/kg. There was no drug-related genetic toxicology finding or tumor formation, or effects on reproduction or development.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data support approval of patisiran-LNP.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

8.1 Pregnancy

Risk Summary

There are no available data on ONPATTRO use in pregnant women to inform a drug-associated risk of adverse developmental outcomes. The effects of a reduction in maternal serum TTR or serum vitamin A levels on the fetus are unknown *[see Clinical Pharmacology (12.2)]*.

(b) (4)

[redacted]

In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2 to 4% and 15 to 20%, respectively. The background risk of major birth defects and miscarriage for the indicated population is unknown.

Data

Animal Data

(b) (4)

[redacted]

8.2 Lactation

Risk Summary

There is no information regarding the presence of ONPATTRO in human milk, the effects on the breastfed infant, or the effects on milk production. The development and health benefits of breastfeeding should be considered along with the mother's clinical need for ONPATTRO and any potential adverse effects on the breastfed infant from ONPATTRO or from the underlying maternal condition.

In lactating rats, patisiran was not present in milk, although small amounts of the lipid components DLin-MC3-DMA and PEG_{2000}-C-DMG were present in milk [redacted] (b) (4)

(b) (4)

[redacted]

12.1 Mechanism of Action

(b) (4)

[redacted]

13 NONCLINICAL TOXICOLOGY

(b) (4)

[redacted]

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

(b) (4)

[redacted]

Mutagenesis

(b) (4)

[redacted]

Impairment of Fertility

(b) (4)

[redacted]

(b) (4)

[redacted]

2 Drug Information

2.1 Drug

CAS Registry Number: N/A

Generic Name: ALN-TTR02, Patisiran-LNP

Code Name: ALN-TTR02

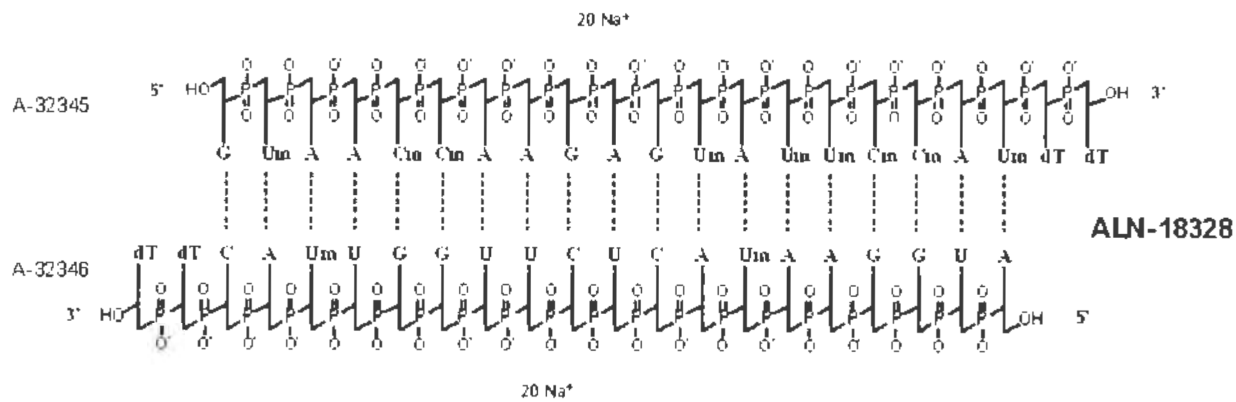
Chemical Name: N/A

Molecular Formula: $C_{412}H_{480}N_{148}Na_{40}O_{290}P_{40}$ (sodium salt)

Molecular Weight: 14303.5 g/mol (sodium salt)

Structure or Biochemical Description:

Figure 1: General Line Diagram for ALN-18328



(Sponsor's Figure)

Pharmacologic Class: siRNA against transthyretin (TTR) mRNA.

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 117395 (ALN-TTR02 for the treatment of TTR-FAP; DNP)

2.3 Drug Formulation

2.0 mg/mL ALN-TTR02 for IV infusion is formulated as lipid nanoparticles in PBS. Novel lipid excipients include DLin-MC3-DMA and PEG_{2000}-C-DMG. Additional excipients include DSPC (1,2-distearolyl-sn-Glycerol-3-phosphocholine) and cholesterol.

Component	Content per Volume (mg/mL)	Content per Vial (mg)	Function	Quality Standard
Patisiran drug substance (patisiran sodium)	2.0 patisiran (equivalent to 2.1 patisiran sodium)	Patisiran 10.0 (equivalent to 10.5 patisiran sodium)	Active ingredient	Manufacturer's specifications
DLin-MC3-DMA	13.0	65.0	(b) (4)	Manufacturer's specifications
PEG_{2000}-C-DMG	1.6	8.0		Manufacturer's specifications
DSPC	3.3	16.5		Manufacturer's specifications
Cholesterol	6.2	31.0		USP/NF, Ph. Eur., JP
PBS[a]				
Sodium phosphate, dibasic, heptahydrate	2.3	11.7		USP, Ph. Eur.
Potassium phosphate, monobasic, anhydrous	0.2	0.9		NF
Sodium chloride	8.8	44.0		USP, Ph. Eur.
Water for injection	qs	qs		USP, Ph. Eur.

[a] values for content per volume have been rounded to two significant figures; content per vial is calculated using non-rounded values

Abbreviations: JP=Japanese Pharmacopoeia; LPN=lipid nanoparticles; NF=National Formulary; PBS=phosphate buffered saline; Ph. Eur.=European Pharmacopoeia; quantum sufficit; USP=United States Pharmacopoeia

(Sponsor's Table)

According to the sponsor, based on a review of the IID, the daily dose of DSPC is below that which would occur following chronic IV administration of other FDA-approved drugs and is, therefore, acceptable.

Based on the proposed dosing regimen for ALN-TTR02, the daily dose of cholesterol is approximately 0.6% of typical circulating endogenous levels. Additionally, administration of ALN-TTR02 in rat and monkey did not result in increases in serum cholesterol levels. The proposed use of cholesterol in the ALN-TTR02 drug product is acceptable.

2.4 Comments on Novel Excipients

As components of the LNP, DLin-MC3-DMA and PEG_{2000}-C-DMG were evaluated in chronic toxicology studies, an in vivo mouse micronucleus assay, and a complete battery of reproductive and developmental toxicity studies. Additionally, DLin-MC3-DMA

and PEG_{2000}-C-DMG were negative when tested directly in Ames and *in vitro* mammalian chromosomal aberration assays. Given these findings, there are no safety concerns regarding the use of DLin-MC3-DMA and PEG_{2000}-C-DMG in the drug product.

2.5 Comments on Impurities/Degradants of Concern

The sponsor identified 8 siRNA-related impurities (DP1 to 8), which consisted of single nucleotide additions or deletions. Such impurities are expected to have minimal pharmacologic activity or be rapidly degraded. Based on discussions with the CMC team, degradation products arising from DLin-MC3-DMA and PEG_{2000}-C-DMG are also of limited concern based on similarity to their respective parent compounds. Impurities arising from the manufacturing process included [redacted] (b) (4) [redacted]). The proposed specification limits for mutagenic impurities are acceptable, and are based on compound-specific acceptable intakes calculated using TD_{50} data from the carcinogenicity potency database (https://toxnet.nlm.nih.gov/cpdb), and adjusted for intermittent, chronic dosing with less-than lifetime exposure.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed dosing for patisiran is 0.3 mg/kg by IV administration once every 3 weeks in patients with hATTR amyloidosis.

2.7 Regulatory Background

Patisiran received fast track designation on October 31, 2013. The requirement for a two-year carcinogenesis study in rat was waived April 20, 2016, based on reduced drug exposure secondary to anti-drug antibodies in the 26-week toxicity study in rats; however, the sponsor was informed that a 6-month study in transgenic TgRasH2 mice would be required at the time of NDA filing. The sponsor was informed in the EOP2 minutes (October 22, 2013) that separate toxicity studies for DLin-MC3-DMA and PEG_{2000}-C-DMG would not be necessary.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

- *In vitro* assessments of target and off-target binding
- *In vitro* suppression of TTR mRNA
- *In vivo* pharmacology in transgenic mice and cynomolgus monkeys

Secondary Pharmacology

- *In vivo* evaluation of serum retinol binding protein

Safety Pharmacology

- *In vitro* effects of the LNP on hERG conductivity
- CNS, cardiovascular, and respiratory safety pharmacology in cynomolgus monkeys

PK/ADME

- Validation of analytical methods
- *In vitro* protein binding
- *In vitro* metabolite profiling
- *In vitro* CYP and drug transporter interactions
- Tissue distribution of radiolabeled ALN-TTR02 in SD rats and cynomolgus monkeys
- PK in SD rats and cynomolgus monkeys
- Metabolite profiling in SD rats
- Excretion mass balance in SD rats

General Toxicology

- Single dose IV injection in cynomolgus monkeys
- SC dosing in SD rats every 2 or 3 weeks for 6 doses
- Once monthly IV infusion in SD rats for 2 doses
- q2w IV infusion in SD rats for 4 doses with 60-day recovery period
- q2w IV infusion for 26-weeks in SD rats with 12-week recovery
- q2w IV infusion in cynomolgus monkeys for 4 doses with 60-day recovery period
- q2w IV infusion in cynomolgus monkeys for 39 weeks with 13-week recovery period

Genotoxicity

- Ames and in vitro chromosome aberration assays for ALN-TTR02, DLin-MC3-DMA, and PEG_{2000}-C-DMG
- Mammalian erythrocyte micronucleus test in mouse bone marrow (ALN-TTR02)

Carcinogenicity

- 26-week IV infusion in TgRasH2 mice

Reproductive and Developmental Toxicity

- Fertility and embryofetal development in SD rats
- Embryofetal development NZW rabbit
- Pre- and postnatal development in SD rats

Other

- Human blood hemolysis

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Review of carcinogenicity range-finding studies and the protocol for the 26-week Tg.RasH2 mouse carcinogenicity study submitted to IND 117395 (David B. Hawver, Ph.D., September 21, 2015)

4 Pharmacology

4.1 Primary Pharmacology

Patisiran-LNP (ALN-TTR02) is a siRNA/lipid nanoparticle (LNP) composition which is targeted to hepatocytes through interactions of the LNP with apolipoprotein E and LDL receptors. The patisiran siRNA component (ALN-18328) suppresses mRNA for WT and mutated TTR, resulting in reductions in circulating levels of total TTR protein. The IC_{50} for TTR mRNA silencing by ALN-18328 in HepG2 and HepB3 cells ranged from 2 to 6 pM. In cynomolgus monkeys, a single 15 min IV infusion of 0.3, 1, or 3 mg/kg ALN-TTR01, which, according to the sponsor, is ALN-18328 formulated with an earlier and less-potent LNP composition, indicated an ED_{50} of 1 mg/kg. A single 15 min IV infusion of 0.03, 0.1, or 0.3 mg/kg ALN-TTR01 in cynomolgus monkeys resulted in reductions in serum TTR of up to 90% and 30% on postdose Days 14 and 28, respectively. TTR tissue deposition studies in transgenic mice (H129-hTTR V30M/Hsf-1 KO) that express human mutant TTR indicated significant reductions in TTR immunoreactivity in esophagus, colon, stomach, sciatic nerve, and dorsal ganglion following six, twice-weekly IV bolus injections of 3 mg/kg ALN-TTR01. Using the proposed ALN-18328/LNP composition (i.e., ALN-TTR02), single IV dosing up to 0.3 mg/kg in cynomolgus monkeys resulted in a 94% decrease in hepatic TTR mRNA, and 80 and 70% decreases in serum TTR protein on Days 14 and 28, respectively. IV infusion of 0.15, 0.2, 0.25 or 0.3 mg/kg ALN-TTR02 monthly or every 3 weeks for 7 or 8 doses resulted in dose-dependent decreases of up to 95% in serum TTR in cynomolgus monkeys, with greater suppression occurring after each dose until the third or fourth dose.

4.2 Secondary Pharmacology

In vitro studies in Hep3B cells indicated a >10,000-fold difference in ALN-18328 binding affinity between on-target and potential off-target transcripts. A non-GLP study evaluating the effects of ALN-TTR01 on retinol binding protein (RBP) was conducted in cynomolgus monkeys (1/sex/group) administered a single 15 min IV infusion of 0 or 3 mg/kg test article; serum TTR and RBP were measured predosing and on Day 7. Reductions in serum TTR of approximately 50% relative to baseline were accompanied by similar reductions in serum RBP. Decreases in serum RBP were likely due to renal excretion in the absence of TTR-binding. Additionally, vitamin A levels were assessed in male and female cynomolgus monkeys administered 0, 0.3, 1, or 3 mg/kg ALN-TTR02 by 1 h IV infusion every 3 weeks for 39 weeks, indicating reductions up to 81% of baseline levels during the dosing period. Because TTR transports thyroxine (T_4), serum T_4 levels were also assessed in the 39-week study; the data indicated up to 50 and 37% reductions in HDM and HDF, respectively, during the dosing period. However, vitamin A and T_4 levels returned to control levels over a 13-week recovery period. Based on the pharmacology studies, off target effects of ALN-TTR02 administration may include decreases in circulating RBP, vitamin A, and T_4 levels.

4.3 Safety Pharmacology

Study title: A Safety Pharmacology Study (with Evaluation of Cardiovascular, Respiratory, and Central Nervous Systems) of ALN-TTR02 Administered to Cynomolgus Monkeys by Intravenous Infusion

Study no.:	TTR02-NCD10-003
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	Not provided; dosing was initiated June 23, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALN-TTR-02, Lot IC-118, 88%

Findings

Phase 1 (Cardiovascular)

Telemetered male cynomolgus monkeys (3/group) were administered a single, 1h IV infusion of 0 (saline) 0.1, 1, 3, or 6 mg/kg ALN-TTR02. There were no drug effects on mean arterial pressure, QTc interval, or ECG rhythm or waveforms. Increases in HR (30 to 120 bpm) in a single animal administered 3 mg/kg began at 240 min postdose and lasted for 48 h. Increases in HR (60 to 120 bpm) in a single animal administered 6 mg/kg began 240 min postdose and lasted for 34 h. Increases in HR were considered by the sponsor to be drug-related.

Phase 2 (CNS and Respiratory)

Three male cynomolgus monkeys were administered a single 1 h IV injection of 3 mg/kg ALN-TTR02. CNS evaluations were conducted prior to initiation of the study and 1 and 24 h postdose; there were no drug effects on behavior, motor function, cranial nerves, or proprioception. Respiratory function was evaluated prior to dosing and 75 min, 5 h, and 25 h postdose; there were no drug effects on respiratory rate, O_2 or CO_2 partial pressures, blood pH, or O_2 saturation.

Study title: Effect of AF-011-1955 on Cloned hERG Potassium Channels Expressed in Mammalian Cells

Study no.:	LD-NCD10-016
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	July 19, 2010
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	AF-011-1955, Lot AP32-03, purity not provided

Findings

Effects of the LNP on hERG conductivity were conducted using a negative control (AF-011-1955) which consists of an siRNA against luciferase incorporated into the LNP used for ALN-TTR02. Concentrations of AF-011-1955 up to 1.5 mg/mL did not inhibit hERG current in transfected HEK cells; an IC_{50} could not be determined.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Validated Analytical Methods

Plasma ALN-18328, DLin-MC3-DMA, and PEG_{2000}-MC3-DMA concentrations in mouse, rat, rabbit, and monkey were quantified using LC/MS/MS assays. Milk concentrations of PEG_{2000}-MC3-DMA and plasma concentrations of AD-18534 (the surrogate siRNA component AF-011-18534) in rat were quantified using LC/MS-HRM assays. Circulating cytokines, anti-PEG IgG and IgM, and indicators of complement activation in monkeys were quantified using ELISA or bead capture (Luminex) assays. Circulating anti-PEG IgG and IgM in rats were quantified using an ELISA assay.

Absorption and Distribution

In vitro incubations of 1.05 mg/mL ALN-TTR02 with human or rat serum albumin, or human α1-acid glycoprotein indicated <2% serum protein binding. A single IV bolus of 0.3 mg/kg ^{14}C-ALN-TTR02 was administered to male SD rats. Maximum radioactivity in the liver (representing 90% of the administered dose) was observed 4 h after dosing.

Metabolism

In vitro incubations of ALN-18328 with serum and S9 fractions from C57Bl/6 mice, SD rats, cynomolgus monkeys, and humans for 24 h indicated comparable degradation by exonuclease cleavage, with up to 23 and 58% degradation of siRNA over 6 and 24 h, respectively.

ALN-18328 and PEG_{2000}-C-DMG were not metabolized by recombinant human CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, or 3A5. However, DLin-MC3-DMA was minimally (up to 26%) metabolized by CYP3A4. *In vivo* studies in rats, monkeys, and humans did not indicate metabolism of PEG_{2000}-C-DMG, while DLin-MC3-DMA was hydrolyzed through an unknown pathway to 4-(dimethylamino)butyric acid (DMBA).

In vitro drug-drug interaction studies indicated that ALN-18238, DLin-MC3-DMA, and PEG_{2000}-C-DMG did not induce CYPs 1A2, 2B6, or 3A4 in isolated human hepatocytes, or inhibit human CYPs 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4/5. The ALN-TTR02 drug product did not inhibit human CYPs 1A1/2, 2C76, 2C43, 2D6, or 3A, or UGT1A1. Additional *in vitro* studies did not indicate inhibition of OATP1B1, OAT1B3, OAT3, OCT2, MATE1, or MATE2 by ALN-18328, DLin-MC3-DMA, or PEG_{2000}-C-DMG.

Excretion
In monkeys, PEG_{2000}-C-DMG and DLin-MC3-DMA (and DMBA) were excreted through the biliary and renal routes, respectively.

6 General Toxicology

6.1 Single-Dose Toxicity

Study title: An Acute Intravenous Injection Toxicity Study of ALN-18328 (With a 14-day Observation Period) in the Cynomolgus Monkey

Study no.: TTR-NCD09-004
Study report location: EDR
Conducting laboratory and location: [redacted] (b) (4)
Date of study initiation: April 3, 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: ALN-18328, Lot GAI-08-130-S7-B1-5.19, 99.8%

Methods

Doses: 0, 10, 30, 100 mg/kg
Frequency of dosing: Single dose
Route of administration: IV bolus
Dose volume: 2 mL/kg
Formulation/Vehicle: Saline
Species/Strain: Cynomolgus monkey
Number/Sex/Group:

Group Number Identification	Dose Level (mg/kg)	Dose Volume (mL/kg)	Animal Numbers			
			Main [a]		Recovery [b]	
			Males	Females	Males	Females
1/ Control	0	2.0	101	151-152	102-103	153
2/ ALN-18328	10	2.0	201-202	251	-	-
3/ ALN-18328	30	2.0	301	351-352	-	-
4/ ALN-18328	100	2.0	401-402	451	403	452-453

a Study animals were euthanized on Day 3.
b Study animals were euthanized on Day 15.

(Sponsor's Table)

Age: 2.5 to 3 years
Weight: 2.3 to 3.1 kg (males), 2.1 to 2.8 kg (females)
Satellite groups: None
Unique study design: None
Deviation from study protocol: No significant deviations

Observations and Results

Mortality and Clinical Signs

Animals were monitored twice daily for mortality or signs of morbidity. Detailed clinical examinations were conducted prior to dosing on Day 1 and weekly thereafter. All animals survived until scheduled necropsy; there were no drug-related clinical signs.

Body Weights and Food Consumption

Body weights were recorded twice weekly. Food consumption was recorded daily. There were no drug effects on body weight or food consumption.

Ophthalmoscopy and ECG

Not evaluated

Hematology, Clinical Chemistry, and Urinalysis

Blood samples from fasted animals were collected predosing and on Days 3 and 15. Urine samples were collected from fasted animals on Days 2 and 14. Serum triglycerides on Day 15 were 2.4-fold lower than baseline in HDM. There were no drug-effects on hematology or urinalysis.

Gross Pathology and Organ Weights

There were no drug-related gross findings or effects on organ weights.

Histopathology

Adequate Battery: Yes

Adrenals	Jejunum	Seminal Vesicles
Aorta	Kidneys	Skeletal Muscle
Bone and Marrow	Liver	Skin
Brain	Lungs	Spinal Cord
Cecum	Lymph Nodes	Spleen
Colon	Mammary Gland	Stomach
Duodenum	Optic Nerves	Testes
Epididymides	Ovaries	Thymus
Esophagus	Pancreas	Thyroid
Eyes	Pituitary	Tongue
Gallbladder	Prostate	Trachea
Heart	Rectum	Urinary Bladder
Ileum	Salivary Gland	Uterus
Injection Sites	Sciatic Nerve	Vagina

Signed Pathology Report: Yes

Peer Review: Yes

Histological Findings: There were no drug-related findings.

Special Evaluation

Complement Factor Analysis (Bb and C4d, CH_{50}):
Blood samples for complement factor analysis were collected predosing and on Day 1 at 15 min and 6, 24, and 48 h postdose; there were no drug effects on circulating complement factors.

Cytokine Stimulation
Blood samples for evaluating IL-1β, IL-6, TNF-α, IFN-α, and IFN-γ stimulation were collected predosing, on Day 1, and at scheduled termination; there were no drug effects.

Toxicokinetics

Not evaluated

Dosing Solution Analysis

Dosing solutions were within 15% of their respective target concentrations.

6.2 Repeat-Dose Toxicity

Study title: Patisiran (ALN-TTTR02): A repeat subcutaneous Dose Tolerability Study in Male Sprague-Dawley Rats

Study no.:	TTR02-NCD14-001
Study report location:	EDR
Conducting laboratory and location:	Alnylam Pharmaceuticals, Inc 300 Third St Cambridge, MA 02142
Date of study initiation:	February 10, 2014
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	ALN-TTR02, Lot L00114,

Findings

Male SD rats (5/group) were administered 0, 0.3, 1, or 10 mg/kg ALN-TTR02 by SC injection once every 2 or 3 weeks for 86 or 128 days, respectively. All HDM were euthanized moribund on Day 3. Clinical signs in the HDM included severe swelling and discoloration at the injection site (9/10), hunched posture (5/10), and mild to severe chromodacryorrhea (6/10). There were no drug-related effects on hematology, clinical chemistry, or coagulation parameters in HDM; gross findings at the injection sites included “clear, firm gelatinous masses” that filled the subcutaneous space, and were characterized histologically by subcutaneous edema and inflammation. Additional histological findings in HDM included minimal to mild macrovesicular hepatocellular

vacuolation in the liver, and marked lymphoid depletion and minimal lymphoid necrosis in the spleen.

All other animals survived until scheduled necropsy. Drug-related clinical signs included injection site wounds/swelling at all doses.

Severity	Wound				Swelling			
	Q2W[a]		Q3W[b]		Q2W[a]		Q3W[b]	
	0.3	1.0	0.3	1.0	0.3	1.0	0.3	1.0
1 (Mild)	7/305 (2.3%)	76/305 (24.9%)	14/450 (3.1%)	84/450 (18.7%)	18/305 (5.9%)	94/305 (30.8%)	41/450 (9.1%)	70/450 (15.6%)
2 (Moderate)	0/305 (0.0%)	15/305 (4.9%)	0/450 (0.0%)	22/450 (4.9%)	28/305 (9.2%)	45/305 (14.8%)	24/450 (5.3%)	24/450 (5.3%)
3 (Severe)	0/305 (0.0%)	9/305 (3.0%)	0/450 (0.0%)	13/450 (2.9%)	12/305 (3.9%)	27/305 (8.9%)	17/450 (3.8%)	30/450 (6.7%)

Note: Incidence is expressed as the number of days with the observation at one or more injection sites/total number of observation days x 5 animals in group.

[a] Q2W = Dosing every 2 weeks for a total of 7 doses. There were 61 observation days for Q2W dosing.

[b] Q3W = Dosing every 3 weeks for a total of 7 doses. There were 90 observation days for Q2W dosing.

(Sponsor's Table)

At terminal necropsy, mean body weights were reduced by 12 and 14% relative to control in Q2W LDM and MDM, respectively, and 7 and 6% in Q3W LDM and MDM, respectively. Drug-related changes in hematology parameters were consistent with the injection site reaction (i.e., increases in mean monocyte and neutrophil counts, and decreases in platelet counts). The MTD for Q2W and Q3W dosing in rats was 0.3 mg/kg.

Compound	Q2W		Q3W	
	C_{max} (µg/mL)	$AUC_{0\text{-}t}$ (µg×h/mL)	C_{max} (µg/mL)	$AUC_{0\text{-}t}$ (µg×h/mL)
ALN-TTR02	0.037	0.66	0.088	1.90
Dlin-MC3-DMA	0.104	2.03	0.104	1.82
PEG_{2000}-C-DMG	0.0500	1.02	0.110	2.08

Systemic exposure at MTD (0.3 mg/kg)

Study title: A 2-Dose (Once Monthly for 2 Months) Intravenous Infusion Toxicity Study of ALN-TTR02 in the Albino Rat

Study no.:	TTR02-NCD11-002
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	May 4, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALN-TTR02, Lot IC118, 87.8%

Methods

Doses:	0, 0.1, 0.3, 1.0 mg/kg
Frequency of dosing:	Once per month
Route of administration:	1 h IV infusion
Dose volume:	12 mL/kg
Formulation/Vehicle:	PBS
Species/Strain:	SD rats
Number/Sex/Group:	10/sex/group
Age:	8 weeks at initiation of dosing
Weight:	252 to 309 g (males), 176 to 214 g (females)
Satellite groups:	TK (12/sex/group)
Unique study design:	None
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality and Clinical Signs

Animals were observed twice daily for mortality or signs of morbidity. Detailed clinical observations were conducted daily. All animals survived until scheduled necropsy. There were no drug-related clinical signs.

Body Weights and Food Consumption

Body weights and food consumption were recorded weekly; there were no drug effects.

Ophthalmoscopy and ECG

Not evaluated

Hematology, Clinical Chemistry, and Urinalysis

Blood samples were collected from fasted animals prior to scheduled necropsy. Urinalysis was not conducted. Drug-related findings in males included dose-dependent decreases in mean platelet counts and increases in APTT, AST, ALT, ALP, TBIL, and urea. Signs of liver injury or thrombocytopenia were not observed in females; however, there was a slight increase in APTT in HDF.

Findings	Sex	Dose (mg/kg)			
		0	0.1	0.3	1.0
Platelets ($10^3/\mu l$)	M	1237.3	1112.4	***983.2****	***955.5****
	F	1181.2	1240.7	1228.9	1151.8
APTT (sec)	M	15.352	15.527	***17.163****	***18.563****
	F	13.877	14.085	14.466	***15.372****
AST (U/L)	M	126.1	223.7	***874.5****	***1759.9****
	F	119	135	127	154
ALT (U/L)	M	39.6	54	***322.7****	***650.1****
	F	31.5	32	29.6	32.2
ALP (U/L)	M	138.1	151.7	***223.8****	***206.7****
	F	91.7	101.3	80.9	76.7
TBIL (mg/dL)	M	0.079	0.088	0.134	***0.183****
	F	0.08	0.076	0.082	0.093
Urea (mg/dL)	M	12.65	14.02	***16.34****	***18.96****
	F	16.01	15.40	13.66	15.96

* indicates statistically significant difference ($p<0.05$) from controls

Gross Pathology and Organ Weights

Pale discoloration of the liver was noted in 1/10 MDM (No. 3006); this finding correlated with hepatocellular necrosis. There were no drug effects on organ weights.

Histopathology

Adequate Battery: Yes

Administration site	Large intestine, cecum
Animal identification	Large intestine, colon
Artery, aorta	Large intestine, rectum
Bone marrow smear	Larynx
Bone marrow, femur	Liver
Bone marrow, sternum	Lung
Bone, femur	Lymph node, mandibular
Bone, sternum	Lymph node, mesenteric
Brain	Nasal cavities
Cervix	Small intestine, duodenum
Epididymis	Small intestine, ileum
Esophagus	Small intestine, jejunum
Eye	Muscle, skeletal
Gland, adrenal	Nerve, optic[a]
Gland, harderian	Nerve, sciatic
Gland, mammary gland	Ovary
Gland lacrimal	Pancreas
Gland, parathyroid	Skin
Gland, pituitary	Spinal cord
Gland, prostate	Spleen
Gland, salivary	Stomach
Gland, seminal vesicle	Testis[b]
Gland, thyroid	Thymus
Gross lesions/masses	Tongue
Gut-associated lymphoid tissue	Trachea
Heart	Urinary bladder
Kidney	Uterus
	Vagina

[a] Preserved in Davidson's fixative.
[b] Preserved in Modified Davidson's fixative.

(Sponsor's Table)

Signed Pathology Report: Yes

Peer Review: Yes

Histological Findings: Minimal bone marrow hypercellularity was observed in HDM; it was unclear if this finding was drug-related. Primary toxicity consisted of liver injury in males. Drug-related spleen atrophy and histiocytosis was present in males and females.

Finding	Males (mg/kg; n= 10/group)				Females (mg/kg; n= 10/group)			
	0.0	**0.1**	**0.3**	**1.0**	**0.0**	**0.1**	**0.3**	**1.0**
Bone Marrow								
Hypercellularity	**0**	**0**	**0**	**3**	**0**	**1**	**0**	**0**
minimal	0	0	0	3	0	0	0	0
slight	0	0	0	0	0	1	0	0
Liver								
Single Cell Necrosis	**0**	**10**	**9**	**9**	**0**	**0**	**2**	**2**
minimal	0	6	6	2	0	0	2	2
slight	0	4	1	3	0	0	0	0
moderate	0	0	3	3	0	0	0	0
marked	0	0	0	1	0	0	0	0
Hepatocellular Necrosis	**0**	**0**	**4**	**7**	**0**	**0**	**0**	**0**
minimal	0	0	1	3	0	0	0	0
slight	0	0	0	1	0	0	0	0
moderate	0	0	3	2	0	0	0	0
marked	0	0	0	1	0	0	0	0
Reactive Sinusoid Lining	**0**	**2**	**6**	**10**	**0**	**0**	**0**	**0**
minimal	0	2	6	4	0	0	0	0
slight	0	0	0	6	0	0	0	0
Hepatocellular Vacuolation	**0**	**0**	**1**	**1**	**2**	**1**	**2**	**1**
minimal	0	0	1	1	2	1	2	2
Spleen								
Atrophy/Necrosis	**0**	**0**	**0**	**5**	**0**	**0**	**0**	**7**
minimal	0	0	0	5	0	0	0	7
Histiocytosis	**0**	**10**	**9**	**9**	**0**	**3**	**5**	**10**
minimal	0	2	9	8	0	3	4	8
slight	0	0	1	1	0	0	1	2

Special Evaluation

Anti-PEG antibodies
Blood samples were collected from main study animals before dosing on Days 14 and 28. Anti-PEG IgM and IgG were detected in 9/60 and 25/60 animals administered ALN-TTR02, respectively.

Toxicokinetics

Following administration of ALN-TTR02, TK for the siRNA component (ALN-18328) were evaluated on Days 1 and 29. There were no clear sex differences in C_{max} or AUC.

Increases in C_{max} and AUC were slightly greater-than dose proportional, and increased with repeat dosing.

Day 1						
ALN-18328						
	Female			Male		
siRNA (mg/kg)	0.1	0.3	1	0.1	0.3	1
Apparent $t_{1/2\beta}$ (hr)	0.44	0.69	NR	NR	1.07	0.56
Tmax (hr)	1.083	1.083	1.083	1.083	1.083	1.083
Cmax (ng/mL)	563	1808	7756	732	2290	9757
AUC_{0-t} (hr·ng/mL)	599	2115	8448	679	2484	9786
$AUC_{0-\infty}$ (hr·ng/mL)	616	2148	NR	NR	2531	9838
CL (mL/hr/kg)	162	140	NR	NR	119	102
Vss (mL/kg)	139	138	NR	NR	112	82.9
AUC_{0-2} (hr·ng/mL)	546	1817	7596	679	2231	8957

Day 29						
ALN-18328						
	Female			Male		
siRNA (mg/kg)	0.1	0.3	1	0.1	0.3	1
Apparent $t_{1/2\beta}$ (hr)	NR	1.13	NR	0.55	0.93	0.84
Tmax (hr)	1.083	1.083	1.083	1.083	1.083	1.083
Cmax (ng/mL)	1172	3673	12990	1313	3240	10790
AUC_{0-t} (hr·ng/mL)	1036	3405	12515	1146	3055	10272
$AUC_{0-\infty}$ (hr·ng/mL)	NR	3454	NR	1169	3108	10349
CL (mL/hr/kg)	NR	86.8	NR	85.5	96.5	96.6
Vss (mL/kg)	NR	72.8	NR	62.7	85.3	78.2
AUC_{0-2} (hr·ng/mL)	997	3167	11976	1100	2777	9452

(Sponsor's Tables)

Dosing Solution Analysis

Dosing solutions ranged from 87.2 to 104% of their respective target concentrations.

Study title: A Multi-dose (Once Bi-weekly x 4 Doses) Study of ALN-TTR02 by Intravenous Infusion in the Albino Rat with a Minimum 60-day Recovery Period

Study no.:	TTR02-NCD10-005
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	October 6, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALN-TTR02, Lot IC118, 88.8%

Methods

Doses:	0, 0.15, 0.8, 1.8, 3 mg/kg ALN-TTR02; 3 mg/kg AF-011-1955
Frequency of dosing:	Once every 2 weeks
Route of administration:	1 h IV infusion
Dose volume:	12 mL/kg
Formulation/Vehicle:	PBS
Species/Strain:	SD rats
Number/Sex/Group:	15/sex/group (ALN-TTR02); 10/sex (AF-011-1955)
Age:	8 to 9 weeks at initiation of dosing
Weight:	266 to 378 g (males), 184 to 252 g (females)
Satellite groups:	TK arm: 14/sex/group (ALN-TTR02), 2/sex/group (PBS and AF-011-1955).
Unique study design:	A comparator group was administered a LNP-packaged siRNA (AF-011-1955) against the luciferase gene to distinguish between siRNA-mediated or lipid nanoparticle toxicities.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality and Clinical Signs

Animals were observed twice daily for mortality or signs of morbidity. Detailed clinical observations were conducted daily. In the TK arm, 3/14 HDM and 1/14 HDF were found dead. Blood collection error was thought to be the COD in 1 HDM; no COD was determined for the remaining deaths. In the main study arm, animal No. 5013 (1.8 mg/kg) was found dead on Day 16. Vascular necrosis and inflammation in the prostate, urinary bladder, and kidneys was thought to be the COD; however, the sponsor did not consider these findings drug-related since they were not present in any other animal at similar or higher doses. There were no drug-related clinical signs in animals that survived until scheduled necropsy.

Body Weights and Food Consumption

Body weights and food consumption were recorded weekly; there were no drug effects.

Ophthalmoscopy

Indirect ophthalmoscopy and slit lamp assessments were conducted predosing and on Day 44; there were no drug-related findings.

ECG

Not evaluated

Hematology, Clinical Chemistry, and Urinalysis

Blood was collected from fasted animals on Days 1, 2, 43, 44, and 104. Urine was collected from fasted animals on Days 42 to 43 and 102 to 103. Drug-related effects on clinical chemistry parameters included elevations in mean ALT and AST in animals dosed with AF-011-1955. Increased mean ALT was observed in HDM and HDF, but were accompanied by high variation and did not reach statistical significance. Elevations in ALT and AST resolved over the recovery period. There were no effects on urinalysis.

Finding	Sex	PBS	AF-011-1955	ALN-TTR02			
		0	3	0.15	0.8	1.8	3.0
Hematology (Day 44)							
Platelets (10^3/L)	M	837.0	***598.4****	994.2	890.9	801.0	730.1
	F	897.1	***758.6****	836.9	943.1	808.2	773.9
Clinical Chemistry (Day 44)							
AST (U/L)	M	172.4	***652.5****	169.9	178.6	188.6	314.7
	F	182.3	269.9	201.2	208.8	182.9	312.2
ALT (U/L)	M	39.7	***329.5****	39.1	40.8	50.9	190.0
	F	52.1	74.5	70.0	111.0	42.9	141.8

* indicates statistically significant difference ($p<0.05$) from controls

<u>Coagulation</u>

3 h after dosing on Day 1, mean APTT was prolonged in males by AF-011-1955. On Day 2, drug effects included prolonged APTT in males administered 0.8, 1.8, or 3.0 mg/kg ALN-TTR02 or 3 mg/kg AF-011-1955, and decreases in fibrinogen in HDM and males administered AF-011-1955. Drug effects on coagulation resolved prior to dosing on Day 43. Possible drug-related decreases in fibrinogen were seen 3 h after administration on Day 43 at all doses in females, but resolved by Day 44; however, a slight increase in APTT was observed in HDF on Day 44.

Gross Pathology and Organ Weights

Gross findings in AF-011-1955 and ALN-TTR02 groups included pale discoloration and enlargement of the adrenal gland and liver, but there was no dose response. Gross findings in the adrenal gland and liver were accompanied by increases in organ weight.

Sex	Males					Females				
Group	3	4	5	6	2	3	4	5	6	2
Test Article	ALN-TTR02				AF-011-1955	ALN-TTR02				AF-011-1955
Dose (mg/kg)	0.15	0.8	1.8	3.0	3.0	0.15	0.8	1.8	3.0	3.0
No. Animals per Group	10	10	10	10	10	10	10	10	10	10
Adrenal										
Absolute value	-3	0	19	**20**	**21**	7	18	10	**13**	17
% of body weight	-2	2	17	20	15	10	19	10	**17**	18
% of brain weight	-5	1	15	19	17	8	18	11	**14**	18
Liver										
Absolute value	4	7	18	**26**	**37**	-11	-5	-4	**10**	5
% of body weight	5	8	**15**	**27**	**29**	-9	-4	-6	**12**	5
% of brain weight	2	7	13	**22**	**33**	-10	-5	-2	**11**	6

[a] All values expressed as percent difference of control group means.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – P ≤ 0.05; refer to data tables for actual significance levels and tests used.

(Sponsor's Table)

Histopathology

Adequate Battery: Yes

Animal identification	Large intestine, cecum
Artery, aorta	Large intestine, colon
Bone marrow smear	Large intestine, rectum
Bone marrow, femur[d]	Larynx
Bone marrow, sternum[d]	Liver
Bone, femur[d]	Lung[c]
Bone, sternum[d]	Lymph node, mandibular
Brain	Lymph node, mesenteric
Cervix	Small intestine, duodenum
Epididymis[b]	Small intestine, ileum
Esophagus	Small intestine, jejunum
Eye[a]	Muscle, skeletal
Gland, adrenal	Nasal cavities[c+d]
Gland, harderian	Nerve, optic[a]
Gland, lacrimal	Nerve, sciatic
Gland, mammary	Ovary
Gland, parathyroid[e]	Pancreas
Gland, pituitary	Skin
Gland, prostate	Spinal cord
Gland, salivary	Spleen
Gland, seminal vesicle	Stomach
Gland, thyroid	Testis[b]
Gross lesions/masses	Thymus
Gut-associated lymphoid tissue	Tongue
Heart	Trachea
Infusion site(s) with catheter tip(s)	Urinary bladder
Kidney	Uterus
	Vagina

a Preserved in Davidson's fixative.
b Preserved in Modified Davidson's fixative.
c Infused with 10% neutral buffered formalin
d Decalcified before sectioning
e Examined only if present in the routine section of thyroid

(Sponsor's Table)

Signed Pathology Report: Yes

Peer Review: Yes

Histological Findings: Target tissues included adrenal gland (cortical hypertrophy), liver (single cell and hepatocellular necrosis, reactive sinusoidal lining, hepatocellular vacuolation, inflammation, and extramedullary hematopoiesis), spleen (lymphoid atrophy/necrosis, histiocytosis, and extramedullary hematopoiesis), testes (degeneration and atrophy of the seminiferous epithelium), epididymis (oligo/aspermia), and infusion site (vascular inflammation).

Sponsor's Table: Histological findings

	Males						Females					
Group	1	2	3	4	5	6	1	2	3	4	5	6
Test Article	PBS Control	AF-011 -1955	ALN-TTR02				PBS Control	AF-011 -1955	ALN-TTR02			
Dose (mg/kg)		3.0	0.15	0.8	1.8	3.0		3.0	0.15	0.8	1.8	3.0
No. Animals per Group	**10**	**10**	**10**	**10**	**11**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Adrenal												
Number Examined	**10**	**10**	**10**	**10**	**11**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Hypertrophy: cortical												
Grade 1	-	3	-	1	2	3	3	2	-	4	3	6
Grade 2	-	-	-	1	-	1	-	2	-	1	-	-
Liver												
Number Examined	**10**	**10**	**10**	**10**	**11**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Necrosis: single cell												
Grade 1	1	3	2	-	2	1	1	6	3	2	3	7
Grade 2	-	5	-	-	-	2	-	1	1	-	-	2
Grade 3	-	1	-	-	-	1	-	-	-	-	-	-
Necrosis: hepatocellular												
Grade 1	-	1	-	-	-	-	-	1	1	-	-	-
Grade 2	-	1	-	-	-	1	-	-	-	1	-	1
Grade 3	-	2	-	-	1	-		-	1	-	-	-
Reactive sinusoidal lining cells												
Grade 1	-	1	5	5	6	1	-	3	5	4	7	4
Grade 2	-	7	-	-	3	8	-	7	2	1	2	2
Grade 3	-	2	-	-	-	1	2	-	-	-	-	2
Grade 4	-	-	-	-	-	-	-	-	-	1	-	1
Vacuolation: hepatocellular												
Grade 1	-	6	5	3	4	2	-	3	2	3	3	4
Grade 2	-	2	1	1	-	4	-	4	1	-	1	-
Grade 3	-	-	-	1	1	3	-	-	-	-	-	-
Grade 4	-	-	-	-	-	1	-	-	-	-	-	-
Inflammation												
Grade 1	-	1	-	-	-	-	-	1	3	-	-	3
Grade 2	-	1	-	-	-	1	-	1	-	-	-	1
Grade 3	-	3	-	-	1	1	-	-	1	1	-	-
Infiltration: mononuclear cell												
Grade 1	-	2	-	-	-	1	2	2	1	2	-	-
Grade 2	-	-	-	-	2	-	-	-	1	1	-	-
Grade 3	-	-	-	-	-	-	-	-	-	-	-	1
Extramedullary hematopoiesis												
Grade 1	-	1	0	1	1	0	1	3	3	2	0	4
Grade 2	-	-	-	-	-	-	1	-	-	-	-	-

Sponsor's Table: Histological findings (Cont.)

	Males						Females					
Group	1	2	3	4	5	6	1	2	3	4	5	6
Test Article	PBS Control	AF-011 -1955	ALN-TTR02				PBS Control	AF-011 -1955	ALN-TTR02			
Dose (mg/kg)		3.0	0.15	0.8	1.8	3.0		3.0	0.15	0.8	1.8	3.0
No. Animals per Group	10	10	10	10	11	10	10	10	10	10	10	10
Spleen												
Number Examined	10	10	10	10	11	10	10	10	10	10	10	10
Atrophy/necrosis: lymphoid												
Grade 1	-	-	3	3	3	1	-	2	1	2	-	3
Grade 2	-	4	3	3	4	2	1	-	-	3	3	1
Grade 3	-	4	-	3	1	5	1	4	1	1	-	2
Grade 4	-	2	-	-	3	2	-	1	-	1	-	1
Histiocytosis												
Grade 1	-	5	1	4	2	4	-	4	1	-	4	6
Grade 2	-	2	-	2	5	5	-	1	-	1	1	2
Extramedullary hematopoiesis												
Grade 1	-	1	2	1	2	2	-	2	-	1	1	2
Grade 2	-	1	-	-	1	-	1	1	1	4	-	1
Grade 3	-	-	-	-	-	-	1	1	-	-	-	-
Testes												
Number Examined	10	10	10	10	10	10	NA	NA	NA	NA	NA	NA
Degeneration/atrophy: seminiferous epithelium							-	-	-	-	-	-
Grade 5	-	-	-	-	1	1	-	-	-	-	-	-
Epididymis												
Number Examined	10	10	10	10	11	10	NA	NA	NA	NA	NA	NA
Oligo/aspermia												
Grade 4	-	-	-	-	1	1	-	-	-	-	-	-
Infusion site												
Number Examined	10	10	10	10	11	10	10	10	10	10	10	10
Inflammation: vascular												
Grade 1	1	-	1	1	4	1	3	2	2	-	1	-
Grade 2	-	-	-	-	-	1	4	3		2	-	-
Grade 3	-	-		1	1	2	-	-	-	1	-	-
Grade 4	-	1	-	-	-	-	-	-	-	1	-	-
Grade 5	-	1	-	-	1	2	-	2		3	-	1
Inflammation: perivascular												
Grade 1	2		2	2	5	1	2	1	3		1	1
Grade 2	-	1	-	-	1	1	3	2	-	2	1	
Grade 3	-	-	-	-	1	3	1	2	-	3	-	2
Grade 4	-	2	1	1	1	-	-	2	-	2	-	2
Grade 5	-	-	-	-	-	1	-	-	-	-	-	-
Bone Marrow												
Number Examined	10	10	10	10	11	10	10	10	10	10	10	10
Hypercellularity: hematopoietic												
Grade 1	-	4	3	2	3	6	2	4	1	3	1	3
Grade 2	-	-	-	-	1	-	-	-	-	-	-	-

Sponsor's Table: Histological findings (Cont.)

	Males						Females					
Group	1	2	3	4	5	6	1	2	3	4	5	6
Test Article	PBS Control	AF-011 -1955	ALN-TTR02				PBS Control	AF-011 -1955	ALN-TTR02			
Dose (mg/kg)		3.0	0.15	0.8	1.8	3.0		3.0	0.15	0.8	1.8	3.0
No. Animals per Group	**10**	**10**	**10**	**10**	**11**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Miscellaneous lymph nodes (mediastinal/tracheobronchial/pancreatic)												
Number Animals Examined	**2**	**5**	**2**	**1**	**2**	**2**	**1**	**4**	**3**	**4**	**0**	**4**
Hyperplasia: lymphoid												
Grade 1	-	-	1	-	-	-	-	3	1	2	-	-
Grade 2	2	-	1	-	1	2	1	-	2	-	-	3
Grade 3	-	1	-	-	-	-	-	-	-	-	-	-
Hyperplasia: stromal cell												
Grade 1	-	1	1	-	-	-	-	3	-	1	-	-
Grade 2	-	-	-	-	-	1	-	-	-	1	-	-
Grade 3	-	-	-	-	-	-	-	-	-	-	-	1
Grade 4	-	1	-	-	-	-	-	-	-	-	-	-
Inflammation												
Grade 1	-	-	-	-	-	-	-	1	-	-	-	-
Grade 2	-	-	-	-	-	-	-	2	-	-	-	1
Grade 3	-	1	-	-	-	1	-	-	-	-	-	-
Histiocytosis												
Grade 1	-	2	-	-	-	-	-	1	-	1	-	1
Grade 2	-	-	-	-	-	-	-	-	-	-	-	1

Special Evaluation

Anti-PEG antibodies

Blood was collected on Days 14 and 42 and assayed for anti-PEG antibodies. Anti-PEG IgG and IgM were found at all ALN-TTR02 doses. There was no dose-response; however, antibody levels were generally higher at Day 14 when compared with Day 42.

Toxicokinetics

TK were evaluated for ALN-18328 (siRNA), DLin-MC3-DMA, and PEG_{2000}-C-DMG on Days 1 and 43. For all compounds, there were no sex differences in plasma C_{max} or AUC, or drug accumulation with repeat dosing. Increases in C_{max} and AUC for all compounds were slightly greater than dose-proportional.

Parameter	Sex	Day 1 (mg/kg)				Day 43 (mg/kg)			
		0.15	0.8	1.8	3	0.15	0.8	1.8	3
ALN-18328									
C_{max} (ng/L)	M	1025	5890	13050	24850	819	4505	18700	35400
	F	1050	7470	14200	25950	1189	1652	15245	32850
AUC_{0-t} (ng×mL/h)	M	1548	6870	21678	36973	N/C	4628	19341	38458
	F	1474	11608	23973	42083	1218	2594	18188	35801
AUC_{0-inf} (ng×mL/h)	M	N/C	6962	21946	37583	N/C	N/C	N/C	N/C
	F	1603	11690	24027	42674	N/C	N/C	18247	35871
DLin-MC3-DMA									
C_{max} (ng/L)	M	8310	40600	105500	152500	4140	29450	110500	248000
	F	6655	46700	109000	199500	7525	9970	93250	229000
AUC_{0-t} (ng×mL/h)	M	20344	106299	310131	603712	21891	122652	280887	623718
	F	19412	118259	279810	484100	20782	77675	257619	695382
AUC_{0-inf} (ng×mL/h)	M	N/C	N/C	N/C	642692	22226	123050	283358	634197
	F	21816	128424	299880	567707	21143	78425	260063	701774
PEG_{2000}-C-DMG									
C_{max} (ng/L)	M	921	4465	11250	23350	802	3595	12200	25950
	F	709	5010	11850	20500	870	2085	11595	24950
AUC_{0-t} (ng×mL/h)	M	2279	13398	36122	77973	2513	16980	35355	54376
	F	1917	14262	34534	61797	2192	12320	35567	57515
AUC_{0-inf} (ng×mL/h)	M	2370	13655	36452	78764	N/C	N/C	N/C	55371
	F	1960	N/C	34960	62262	2408	13657	N/C	N/C

(Plasma TK; N/C = not computed)

Dosing Solution Analysis

Dosing solutions were within 15% of their respective target concentrations.

Study title: ALN-TTR02: A 26-Week Chronic Toxicity and Toxicokinetic Study in Sprague Dawley Rats with a 12-Week Recovery Period

Study no.:	TTR02-NCD12-003
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	June 1, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALN-TTR02, Lot L00114, 91.7%

Methods

Doses:	0, 0.03, 0.1, 0.3 mg/kg
Frequency of dosing:	Once every 2 weeks
Route of administration:	1 h IV infusion
Dose volume:	12 mL/kg/h
Formulation/Vehicle:	Saline
Species/Strain:	SD rats
Number/Sex/Group:	15/sex group (main); 10/sex/group (recovery)
Age:	9 to 10 weeks
Weight:	281 to 450 g (males), 176 to 274 g (females)
Satellite groups:	TK arm (21/sex/group)
Unique study design:	None
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality and Clinical Signs

Animals were observed twice daily for mortality or signs of morbidity. Detailed clinical evaluations were conducted weekly. Five animals in the main group and 6 in the recovery group were either found dead or euthanized moribund. However, COD was thought to be due to complications with indwelling catheters. Additionally, 13 TK animals were either found dead or euthanized moribund, but the COD was thought to be related to jugular venipuncture. There were no drug-related clinical signs.

Body Weights and Food Consumption

Body weights and food consumption were recorded weekly. There were no drug-related effects on mean weight gain or food consumption.

Ophthalmoscopy

Indirect ophthalmoscopy and slit lamp biomicroscopy were conducted predosing and at the end of the dosing and recovery periods. There were no drug-related findings.

ECG

Not evaluated

Hematology, Clinical Chemistry and Urinalysis

Blood and urine samples were collected from fasted animals prior to scheduled necropsy. Dose-dependent decreases in mean reticulocyte counts were observed in MDM and HDM; increases in fibrinogen were observed in MDF and HDF. Both findings resolved over the recovery period. There were no drug effects on clinical chemistry or urinalysis parameters.

Finding	Sex	Main (mg/kg)				Recovery (mg/kg)			
		0	0.03	0.1	0.3	0	0.03	0.1	0.3
Reticulocytes (10^9/L)	M	300.6	271.4	***218.49****	***179.51****	245.2	302.1	270.3	203.2
	F	292.85	238.95	231.86	271.22	153.7	157.8	220.7	147.7
Fibrinogen (mg/dL)	M	226.8	226.6	234.7	257.7	172.0	171.9	185.5	166.1
	F	167.7	177.5	***199.9****	***219.5****	139.6	141.1	135.0	126.3

* indicates statistically significant difference (*p<0.05*) from controls

Gross Pathology and Organ Weights

Non-neoplastic masses were observed at the infusion site in all groups; masses were characterized histologically by vascular/perivascular inflammation. There were no drug effects on organ weights.

Summary of Gross Pathology Findings at the Infusion Site – Scheduled Euthanasia (Day 186)

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Test Article	**RI[a]**	**ALN-TTR02**			**RI[a]**	**ALN-TTR02**		
Dose (mg/kg/dose)	**0**	**0.03**	**0.1**	**0.3**	**0**	**0.03**	**0.1**	**0.3**
No. Animals per Group	**15**	**14**	**15**	**15**	**13**	**15**	**14**	**14**
Infusion site (No. Examined)	15	14	15	15	13	15	14	14
Mass	9	7	10	14	5	8	8	11

[a] Reference item.

(Sponsor's Table)

Histopathology

Adequate Battery: Yes

Animal identification	Large intestine, cecum
Artery, aorta	Large intestine, colon
Bone marrow smear	Large intestine, rectum
Bone marrow, femur	Larynx
Bone marrow, sternum	Liver
Bone, femur	Lung
Bone, sternum	Lymph node, mandibular
Brain	Lymph node, mesenteric
Cervix	Small intestine, duodenum
Epididymis [b]	Small intestine, ileum
Esophagus	Small intestine, jejunum
Eye [a]	Muscle, skeletal
Gland, adrenal	Nasal Cavities
Gland, harderian	Nerve, optic[a]
Gland, lacrimal	Nerve, sciatic
Gland, mammary gland	Ovary
Gland, parathyroid	Pancreas
Gland, pituitary	Skin
Gland, prostate	Spinal cord
Gland, salivary	Spleen
Gland, seminal vesicle	Stomach
Gland, thyroid	Testis[b]
Gross lesions/masses	Thymus
Gut-associated lymphoid tissue	Tongue
Heart	Trachea
Infusion site with catheter tip	Urinary bladder
Infusion site via tail vein(s) (as applicable)	Uterus
Kidney	Vagina

[a] Preserved in Davidson's fixative.
[b] Preserved in Modified Davidson's fixative.

(Sponsor's Table)

Signed Pathology Report: Yes

Peer Review: Yes

Histological Findings:
Drug-related findings included vascular/perivascular inflammation at the infusion site in all groups, and hepatic periportal vacuolation in HDM and HDF. According to the sponsor, the infusion site inflammation was characterized by "*necrotic material surrounded by a thick fibrous capsule (consistent with abscess formation) associated with edema and infiltrates of macrophages, lymphocytes, and/or neutrophils.*" Liver and infusion site pathology resolved over the recovery period.

Finding	**Males (mg/kg)**				**Females (mg/kg)**			
	0.0	**0.03**	**0.1**	**0.3**	**0.0**	**0.03**	**0.1**	**0.3**
	n=15	**n=13**	**n=15**	**n=15**	**n=13**	**n=15**	**n=14**	**n=14**
Infusion Site								
Inflammation	**15**	**13**	**15**	**15**	**13**	**15**	**14**	**14**
minimal	0	2	0	0	1	4	1	0
slight	1	1	1	0	4	3	2	2
moderate	13	8	8	8	6	5	7	6
marked	0	2	4	4	2	2	2	3
severe	1	0	2	3	0	1	2	3
Liver								
Hepatocyte Vacuolation	**2**	**2**	**2**	**9**	**3**	**5**	**5**	**6**
minimal	1	2	2	8	2	2	4	2
slight	1	0	0	1	1	3	1	3
moderate	0	0	0	0	0	0	0	1

Special Evaluation

Anti-PEG antibodies

Blood samples for anti-PEG antibodies were collected on Days 15, 183, and 267. During the dosing period, ADA were present in 46 to 49% of the low, mid, and high-dose animals. ADA formation was not dose- or sex-dependent.

Toxicokinetics

Plasma C_{max} and AUC for ALN-18328, DLin-MC3-DMA, and $PEG2_{000}$-C-DMG were slightly higher in males than females. Increases in C_{max} and AUC for ALN-18328 were slightly greater than dose proportional. Increases in C_{max} and AUC for DLin-MC3-DMA and $PEG2_{000}$-C-DMG were greater than dose-proportional. Decreases in ALN-18328 exposure on Day 183 were thought by the sponsor to be secondary to anti-drug antibodies.

Parameter	Sex	Day 1 (mg/kg)			Day 183 (mg/kg)		
		0.03	0.1	0.3	0.03	0.1	0.3
ALN-18328							
C_{max} (ng/L)	M	231	1250	3300	N/A	N/A	132
	F	30.8	1040	1510	N/A	667	N/A
$AUC_{0\text{-}last}$ (ng×mL/h)	M	N/A	2350	4210	N/A	N/A	N/A
	F	N/A	1590	2090	N/A	N/A	N/A
$AUC_{0\text{-}inf}$ (ng×mL/h)	M	N/A	2590	4380	N/A	N/A	N/A
	F	N/A	1650	N/A	N/A	N/A	N/A
DLin-MC3-DMA							
C_{max} (ng/L)	M	812	8090	24600	40.5	36.3	1670
	F	147	5000	11400	13.1	4600	277
$AUC_{0\text{-}last}$ (ng×mL/h)	M	3710	28500	86700	4550	13300	65000
	F	1360	14300	40300	2090	15100	39400
$AUC_{0\text{-}inf}$ (ng×mL/h)	M	3930	29300	89900	4830	13800	N/A
	F	1510	16400	45100	2340	15200	N/A
PEG_{2000}-C-DMG							
C_{max} (ng/L)	M	107	1050	2880	20.4	37.3	544
	F	38.7	890	1600	5.45	483	196
$AUC_{0\text{-}last}$ (ng×mL/h)	M	259	2770	8860	74.7	938	7810
	F	121	2280	4800	10.9	1090	6040
$AUC_{0\text{-}inf}$ (ng×mL/h)	M	282	2850	8990	N/A	1110	9620
	F	N/A	N/A	4970	N/A	N/A	9650

Dosing Solution Analysis

Dosing solutions were within 15% of their respective target concentrations.

Study title: A Multi-dose (Once Bi-weekly x 4 Doses) Study of ALN-TTR02 by Intravenous Infusion in the Cynomolgus Monkey with a 60-day Recovery Period.

Study no.:	TTR02-NCD10-011
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	October 18, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALN-TTR02, Lot IC118, 87.8% AF-01101955, Lot IC116, 88.8%

Methods

Doses:	0, 0.3, 1.0, 3 mg/kg ALN-TTR02 (main and recovery); 3 mg/kg AF-011-1955 (main)
Frequency of dosing:	Every 2 weeks
Route of administration:	1 h IV infusion
Dose volume:	20 mL/kg
Formulation/Vehicle:	PBS
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	3/sex/group (main); 3/sex/group (recovery)
Age:	3 to 4 years
Weight:	2.8 to 4.2 kg (males), 2.7 to 3.2 (females)
Satellite groups:	TK arm
Unique study design:	A comparator group was administered a lipid-packaged siRNA (AF-011-1955) against the luciferase gene to distinguish between siRNA-mediated or lipid nanoparticle toxicities.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality and Clinical Signs

Animals were observed twice daily for mortality or signs of morbidity. Detailed clinical observations were conducted weekly. All animals survived until scheduled necropsy. There were no drug-related clinical signs.

Body Weights and Food Consumption

Body weights were recorded weekly. Qualitative evaluations of food intake were conducted daily. There were no drug effects on body weight gain or food consumption.

Ophthalmoscopy

Examinations by direct and indirect ophthalmoscopy and slit lamp biomicroscopy were conducted predosing and on Days 44 and 104. There were no drug effects.

ECG

Not evaluated

Hematology, Clinical Chemistry, and Urinalysis

Blood samples were collected from fasted animals predosing and on Days 2, 42, 44, 70, and 104. Urine samples were collected predosing and on Days 3, 45, and 104. There were no drug effects on hematology, coagulation, or urinalysis parameters. Elevations in mean AST and ALT were observed following dosing with AF-011-1955 or ALN-TTR02 but generally resolved during the two-week period between dosing. It was unclear why mean AST values were elevated in control animals after dosing. There were no drug-related findings in the recovery group.

Finding	Day	Males (mg/kg)					Females (mg/kg)				
		PBS	AF-011-1955	ALN-TTR02			PBS	AF-011-1955	ALN-TTR02		
		0	3.0	0.3	1.0	3.0	0	3.0	0.3	1.0	3.0
AST (U/L)	Pre	52.3	53.7	47.8	46.0	48.2	45.8	47.3	39.2	47.7	45.2
	2	124.8	308.7	266.7	132.3	250.2	140.2	170.7	132.5	148.5	118.2
	42	49.3	73.3	50.8	55.8	60.2	45.0	59.0	39.3	54.3	61.0
	44	132.3	344.7	239.3	109.0	185.5	125.0	287.0	109.8	260.3	135.7
	70	54.3	--	42.3	44.7	51.3	35.3	--	45.7	51.7	64.0
	104	68.7	--	63.7	61.7	71.7	88.0	--	61.7	69.0	75.0
ALT (U/L)	Pre	43.3	54.0	52.7	41.5	48.5	59.3	51.7	45.8	44.7	44.8
	2	85.2	***293.0****	143.5	74.0	234.0	119.3	207.0	122.7	90.5	132.7
	42	37.3	***82.7****	49.7	40.3	***68.3****	54.3	100.3	37.8	60.8	70.5
	44	61.2	***207.3****	103.2	57.5	122.0	99.8	259.3	66.0	109.3	89.0
	70	33.3	--	39.3	41.3	48.0	43.3	--	38.3	48.7	51.7
	104	45.7	--	45.3	42.7	44.0	52.3	--	55.7	46.3	41.3

Gross Pathology and Organ Weights

Drug-related gross findings included liver discoloration and small spleen size. There were no drug-related changes in organ weights.

Finding	Males (mg/kg)					Females (mg/kg)				
	PBS	AF-011-1955	ALN-TTR02			PBS	AF-011-1955	ALN-TTR02		
	0	3.0	0.3	1.0	3.0	0	3.0	0.3	1.0	3.0
Main Study										
Liver Discoloration	0/3	2/3	0/3	0/3	1/3	0/3	2/3	0/3	0/3	0/3
Small Spleen	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
Recovery										
Small Spleen	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3

Histopathology

Adequate Battery: Yes

Infusion sites	Large intestine, colon
Animal identification	Large intestine, rectum
Artery, aorta	Larynx
Bone marrow smear	Liver
Bone marrow, femur	Lung
Bone, femur	Lymph node, mandibular
Bone, sternum	Lymph node, mesenteric
Brain	Small intestine, duodenum
Cervix	Small intestine, ileum
Epididymis[b]	Small intestine, jejunum
Esophagus	Muscle, skeletal
Eye[a]	Nerve, optic[a]
Gallbladder	Nerve, sciatic
Gland, adrenal	Ovary
Gland, mammary	Pancreas
Gland, parathyroid	Skin
Gland, pituitary	Spinal cord
Gland, prostate	Spleen
Gland, salivary	Stomach
Gland, seminal vesicle	Testis[b]
Gland, thyroid	Thymus
Gross lesions/masses	Tongue
Gut-associated lymphoid tissue	Trachea
Heart	Urinary bladder
Kidney	Uterus
Large intestine, cecum	Vagina

[a] Preserved in Davidson's fixative.
[b] Preserved in Modified Davidson's fixative.

(Sponsor's Table)

Signed Pathology Report: Yes

Peer Review: Yes

Histological Findings: Liver findings included centrilobular vacuolation, single cell necrosis, and reactive sinusoidal cells. Spleen findings included hypocellularity of the red pulp. Adrenal gland findings included decreased cortical vacuolation. Infusion site findings included perivascular inflammation. Histology findings after the recovery period

included minimal hepatic pigment deposition in HDM (2/3) and HDF (1/3) and minimal splenic red pulp hypocellularity in HDF (1/3).

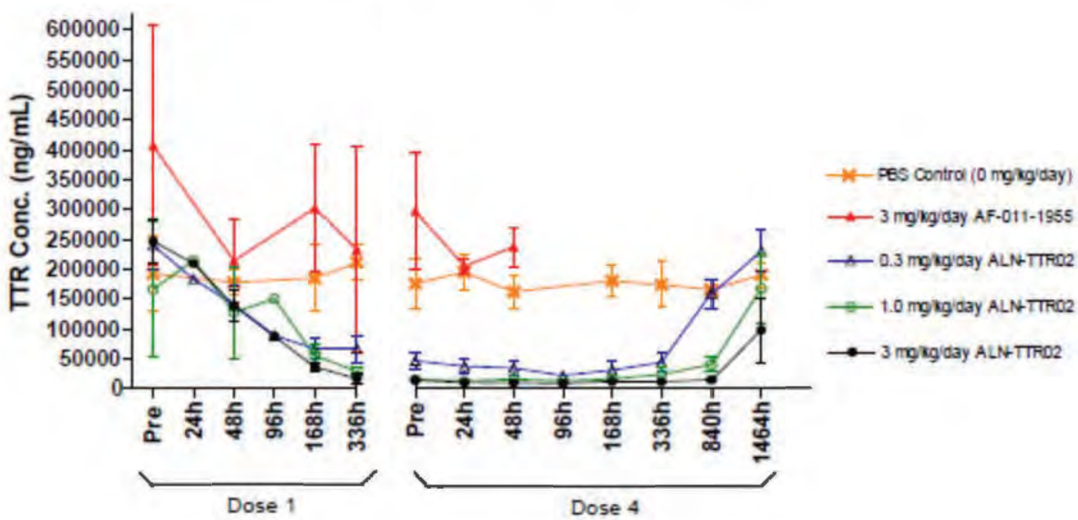
Finding	Males (mg/kg; n=3)					Females (mg/kg; n=3)				
	PBS	AF-011-1955	ALN-TTR02			PBS	AF-011-1955	ALN-TTR02		
	0	3.0	0.3	1.0	3.0	0	3.0	0.3	1.0	3.0
Liver										
Centrilobular Hepatocellular Vacuolation	**0**	**3**	**0**	**0**	**3**	**0**	**3**	**0**	**0**	**3**
minimal	0	0	0	0	0	0	0	0	0	1
slight	0	2	0	0	1	0	1	0	0	1
moderate	0	1	0	0	2	0	2	0	0	1
Single Cell Necrosis	**0**	**3**	**0**	**0**	**3**	**0**	**3**	**0**	**0**	**2**
minimal	0	2	0	0	1	0	1	0	0	0
slight	0	0	0	0	2	0	1	0	0	1
moderate	0	1	0	0	0	0	1	0	0	1
Reactive Sinusoid	**0**	**2**	**0**	**0**	**3**	**0**	**2**	**0**	**0**	**2**
minimal	0	1	0	0	2	0	1	0	0	1
slight	0	1	0	0	1	0	1	0	0	1
Infiltration	**0**	**3**	**0**	**0**	**3**	**0**	**3**	**0**	**0**	**2**
minimal	0	1	0	0	2	0	1	0	0	2
slight	0	2	0	0	1	0	2	0	0	0
Pigment Deposit	**0**	**3**	**0**	**0**	**3**	**0**	**3**	**0**	**0**	**2**
minimal	0	0	0	0	2	0	1	0	0	1
slight	0	3	0	0	1	0	2	0	0	1
Spleen										
Red Pulp Hypocellularity	**0**	**1**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**
minimal	0	1	0	0	0	0	1	0	0	0
slight	0	0	0	0	0	0	0	0	0	0
moderate	0	0	0	0	0	0	1	0	0	0
Adrenal Gland										
↓ Cortical Vacuolation	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**2**
minimal	0	0	0	0	1	0	0	0	0	2
Infusion Site										
Perivascular Inflammation	**2**	**2**	**3**	**0**	**2**	**1**	**3**	**2**	**2**	**3**
minimal	2	1	3	0	1	1	0	2	2	1
slight	0	1	0	0	0	0	3	0	0	2
moderate	0	0	0	0	1	0	0	0	0	0

Special Evaluation

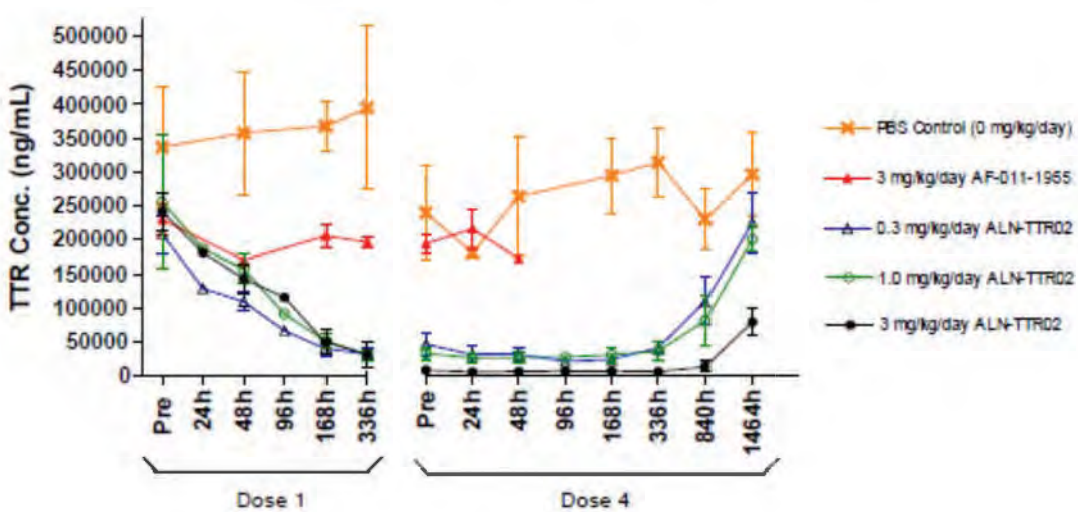
Immunochemistry (T_3 and total T_4)
There were no drug effects on T_3. Total T_4 levels were decreased up to 41% after the 4th dose in LDM and from 28 to 46% in MDM and HDM following the 1st dose. Transient, minimal decreases in total T_4 were observed at all doses in females. Total T_4 levels had normalized at all doses by 60 days after the 4th dose. Decreases in T_4 were thought to be due to reductions in TTR.

Liver TTR mRNA Levels and Serum TTR Concentrations
99% suppression of liver TTR mRNA was achieved by Day 45 at all ALM-TTR02 doses. Maximal inhibition of serum TTR protein levels were achieved approximately 14 days after the initial dose and were maintained until approximately 14 to 35 days following the 4th dose.

Text Figure 1
Mean Change in Serum TTR Concentration Over Time (Males)



Text Figure 2
Mean Change in Serum TTR Concentration Over Time (Females)



(Sponsor's Figure)

Serum Vitamin A Levels

Dose-dependent decrease in vitamin A levels were observed following the first dose and were sustained throughout the dosing period. Vitamin A levels had generally returned to control levels within 5 weeks following the final dose of 0.3 or 1 mg/kg and were trending toward control levels in animals dosed with 3 mg/kg.

Anti-PEG Antibodies

A non-dose-dependent increase in anti-PEG IgM and IgG was observed in all ALN-TTR02 and AF-01101955 groups by Day 14. Anti-PEG IgM levels had resolved in all

animals by the 4th dose, while IgG levels were resolved in 9/14 animals. There were no signs of a memory response with repeat dosing.

Complement Split Products (Bb and C3a)
Increases in Bb and C3a were observed at all doses but were not dose-dependent. The incidence of elevated Bb or C3a was greater in females.

Finding	Day	Males (mg/kg)					Females (mg/kg)				
		PBS	AF-011-1955	ALN-TTR02			PBS	AF-011-1955	ALN-TTR02		
		0	3.0	0.3	1.0	3.0	0	3.0	0.3	1.0	3.0
↑Bb (incidence)	1 (pre)	0/6	0/3	0/6	1/6	0/6	1/6	0/3	0/6	0/6	0/6
	1 (+0.25 h)	0/6	0/3	0/6	2/6	1/6	1/6	1/3	2/6	6/6	1/6
	1 (+6 h)	1/6	2/3	2/6	2/6	2/6	6/6	2/3	1/6	6/6	4/6
	2	0/6	1/3	1/6	1/6	2/6	1/6	2/3	0/6	2/6	2/6
	43 (pre)	0/6	0/3	0/6	1/6	2/6	0/6	1/3	0/6	1/6	2/6
	43 (+0.25 h)	0/6	0/3	2/6	3/6	4/6	0/6	1/3	4/6	5/6	5/6
	43 (+6 h)	0/6	3/3	2/6	3/6	6/6	3/6	3/3	4/6	5/6	5/6
	44	0/6	2/3	0/6	1/6	4/6	0/6	2/3	0/6	3/6	3/6
	104	0/3	--	0/3	0/3	0/3	0/3	--	0/3	0/3	0/3
↑C3a (incidence)	1 (pre)	0/6	1/3	0/6	2/6	0/6	0/6	0/3	0/6	2/6	0/6
	1 (+0.25 h)	0/6	2/3	0/6	2/6	1/6	0/6	1/3	1/6	4/6	2/6
	1 (+6 h)	0/6	2/3	0/6	1/6	1/6	2/6	1/3	0/6	2/6	2/6
	2	0/6	1/3	0/6	0/6	3/6	1/6	1/3	0/6	2/6	4/6
	43 (pre)	0/6	1/3	0/6	0/6	0/6	2/6	2/3	2/6	1/6	0/6
	43 (+0.25 h)	0/6	2/3	0/6	1/6	1/6	1/6	2/3	0/6	3/6	2/6
	43 (+6 h)	0/6	3/3	0/6	0/6	1/6	2/6	2/3	0/6	1/6	1/6
	44	0/6	1/3	1/6	0/6	0/6	2/6	2/3	0/6	3/6	1/6
	104	0/3	--	0/3	0/6	0/3	0/3	--	0/3	1/3	0/3

Cytokine Stimulation
IL-1β concentrations were below the limit of quantitation at all timepoints. IL-1RA and IL-6 levels were also generally below the limit of quantitation; however, transient elevations were observed after dosing. There were no drug-effects effects on IFN-γ, TNF-α, IFN-α, or CRP.

Finding	Day	Males (mg/kg)					Females (mg/kg)				
		PBS	AF-011-1955	ALN-TTR02			PBS	AF-011-1955	ALN-TTR02		
		0	3.0	0.3	1.0	3.0	0	3.0	0.3	1.0	3.0
↑IL-1RA (incidence)	1 (pre)	--	--	--	--	--	--	--	--	--	--
	1 (+3 h)	0/6	3/3	1/6	3/6	6/6	0/6	3/3	1/6	2/6	2/6
	2	--	--	--	--	--	--	--	--	--	--
	43 (pre)	--	--	--	--	1/6	--	--	--	--	--
	43 (+3 h)	1/6	3/3	0/6	0/6	4/6	0/6	2/3	0/6	3/6	3/6
	44	--	--	--	--	--	--	--	--	--	--
	104	--	--	--	--	--	--	--	--	--	--
↑IL-6 (incidence)	1 (pre)	--	--	--	--	--	--	--	--	--	--
	1 (+3 h)	1/6	1/3	0/6	0/6	0/6	0/6	3/3	--	1/6	1/6
	2	--	--	--	--	--	--	--	--	--	--
	43 (pre)	1/6	--	--	--	1/6	--	--	--	--	--
	43 (+3 h)	1/6	1/3	1/6	1/6	4/6	1/6	1/3	2/6	0/5	1/6
	44	--	--	--	--	--	--	--	--	--	--
	104	--	--	--	--	--	--	--	--	--	--

Toxicokinetics

TK analysis was conducted on Days 1 and 43. Increases in plasma ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG C_{max} and AUC were general dose-proportional and did not increase with repeat dosing.

Mean Toxicokinetic Parameters of ALN-18328 after a 1-hour IV Infusion of ALN-TTR02 to Cynomolgus Monkeys

	Day 1			Day 43		
Sex	Male					
ALN-TTR02 (mg/kg)	0.3	1	3	0.3	1	3
Apparent $t_{1/2\beta}$ (hr)	7.19	3.99	9.37	9.63	8.04	13.3
Tmax (hr)	0.50	1.083	1.083	1.083	1.083	1.083
Cmax (ng/mL)	2920	16200	56700	4215	16850	77900
AUC_{0-25} (hr·ng/mL)	5025	34516	130962	8493	32698	161169
Sex	Female					
Apparent $t_{1/2\beta}$ (hr)	10.2	8.15	12.3	16.1	13.4	17.0
Tmax (hr)	0.79	1.083	1.083	0.79	1.083	1.083
Cmax (ng/mL)	2950	13250	67700	2395	19900	62400
AUC_{0-25} (hr·ng/mL)	7720	38920	169522	6293	45733	120705

AUC_{0-25} was used for comparison of total exposure on Day 1 and 43.

Mean Toxicokinetic Parameters of DLin-MC3-DMA after a 1-hour IV Infusion of ALN-TTR02 to Cynomolgus Monkeys

	Day 1			Day 43		
Sex	Male					
ALN-TTR02 (mg/kg)	0.3	1	3	0.3	1	3
DLin-MC3-DMA (mg/kg)	2.03	6.76	20.3	2.03	6.76	20.3
Apparent $t_{1/2\beta}$ (hr)	560	506	NC	963	934	988
Tmax (hr)	0.79	1.083	1.083	1.083	1.083	1.083
Cmax (ng/mL)	29150	118500	486000	33250	122250	479000
$AUC_{0\text{-}337}$ (hr·ng/mL)	360248	962744	4145292	436705	1149492	4552791
Sex	Female					
Apparent $t_{1/2\beta}$ (hr)	277	623	222	512	1044	721
Tmax (hr)	0.79	1.083	1.083	0.79	1.083	1.50
Cmax (ng/mL)	23800	121000	582500	15800	142500	279000
$AUC_{0\text{-}337}$ (hr·ng/mL)	336700	1034176	3969661	366349	1194359	3214912

$AUC_{0\text{-}337}$ was used for comparison of total exposure on Day 1 and 43.
NC = Not calculable.

Mean Toxicokinetic Parameters of PEG2000-C-DMG after a 1-hour IV Infusion of ALN-TTR02 to Cynomolgus Monkeys

	Day 1			Day 43		
Sex	Male					
ALN-TTR02 (mg/kg)	0.3	1	3	0.3	1	3
PEG_{2000}-C-DMG (mg/kg)	0.23	0.76	2.29	0.23	0.76	2.29
Apparent $t_{1/2\beta}$ (hr)	92.9	95.6	74.0	81.3	326	211
Tmax (hr)	1.083	1.083	1.083	1.54	2.04	1.083
Cmax (ng/mL)	3895	16400	53750	4975	16700	61850
$AUC_{0\text{-}337}$ (hr·ng/mL)	76664	259423	965445	76337	299823	879846
Sex	Female					
Apparent $t_{1/2\beta}$ (hr)	95.9	77.3	79.9	91.8	158	165
Tmax (hr)	1.29	1.083	1.083	2.50	1.083	1.083
Cmax (ng/mL)	4185	16750	55450	3535	16200	51500
$AUC_{0\text{-}337}$ (hr·ng/mL)	58424	217198	611991	58359	249335	472071

$AUC_{0\text{-}337}$ was used for comparison of total exposure on Day 1 and 43.

(Sponsor's Tables)

Dosing Solution Analysis

Dosing solutions were within 15% of their respective target concentrations.

Study title: A 39-Week Chronic Toxicity and Toxicokinetic Study in Cynomolgus Monkeys with a 13-Week Recovery Period

Study no.:	TTR02-NCD12-001
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	March 12, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALN-TTR02, IC118, 87.8%

Methods

Doses:	0, 0.3, 1, or 3/2 mg/kg (see deviations)
Frequency of dosing:	Once every 3 weeks
Route of administration:	1 h IV infusion
Dose volume:	20 mL/kg
Formulation/Vehicle:	PBS
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	3/sex/group (main); 3/sex/group (recovery)
Age:	5 to 6 years
Weight:	4.5 to 6.3 kg (males); 2.6 to 3.7 kg (females)
Satellite groups:	TK
Unique study design:	None
Deviation from study protocol:	After Day 22, the HD was reduced from 3 mg/kg to 2 mg/kg based on elevations in liver function tests.

Observations and Results

Mortality and Clinical Signs

Animals were monitored twice daily for mortality or signs of morbidity. Detailed examinations were conducted once weekly. One HDF (No. 4506) was found dead on Day 23 following a dose of 2 mg/kg on Day 22; however, a COD was not determined. There were no drug-related clinical signs in any group.

Body Weights and Food Consumption

Body weights were recorded weekly. Qualitative food consumption was evaluated daily. There were no drug-effects on body weights or food consumption.

Ophthalmoscopy

Examinations by direct and indirect ophthalmoscopy and slit lamp biomicroscopy were conducted predosing and on Weeks 21, 39 and 52. Electroretinography was conducted predosing and during Weeks 20, 38, and 51. There were no drug-related findings.

ECG

Not evaluated

Hematology, Clinical Chemistry, and Urinalysis

Blood and samples were collected from fasted animals predosing and on Days 4, 22, 25, 43, 88, 172, 277, and 365. Urine samples were collected from predosing and on Days 3, 87, 171, 276, and 364. There were no drug effects on hematology, coagulation, or urinalysis parameters. Drug-related effects on clinical chemistry parameters included elevations in liver function tests (LFT) in HDM. Drug-related increases in LFT were maximal 4 days after the initial dose. Subsequent doses resulted in less pronounced elevations in LFT. Although elevations in LFT generally resolved by the end of the recovery period, slight elevations in mean ALT and AST were observed on Day 365 in HDM; however, this observation was thought to be due to a single animal that was receiving Caprofen® and enrofloxacin after a wound repair surgery.

Finding	Day	Males (mg/kg)				Females (mg/kg)			
		0	0.3	1.0	3.0	0	0.3	1.0	3.0
AST (U/L)	Pre	46.0	39.5	45.5	55.2	33.3	36.2	36.8	34.7
	4	67.7	33.0	50.0	1411.3	51.5	36.8	34.5	94.5
	22	37.7	30.2	35.5	38.3	33.0	32.0	30.5	37.5
	88	48.5	29.7	49.3	103.2	34.8	31.5	30.8	45.4
	172	60.8	29.0	45.7	100.8	40.5	31.3	28.5	54.0
	277	54.8	34.3	47.7	132.2	40.3	38.0	30.5	44.2
	365	34.7	31.0	39.3	86.7	37.7	31.0	31.3	32.0
ALT (U/L)	Pre	55.8	34.7	37.7	44.7	35.3	52.5	37.3	41.8
	4	79.0	45.7	48.2	2087.0	63.0	63.5	49.5	286.3
	22	43.0	28.0	28.5	46.7	31.8	45.2	33.0	39.2
	88	68.7	33.7	41.8	228.0	54.5	52.0	45.7	85.8
	172	80.3	32.7	46.0	149.0	64.8	54.3	43.0	104.2
	277	76.5	46.5	50.5	212.7	60.3	74.3	46.2	73.8
	365	57.3	38.7	36.3	86.0	41.0	62.3	44.7	38.5
AP (U/L)	Pre	274.7	259.0	283.3	337.2	145.7	195.7	191.2	226.5
	4	261.3	223.2	290.0	623.2	147.3	198.0	200.8	279.0
	22	277.2	226.8	298.3	426.0	151.3	186.7	203.5	265.7
	88	271.5	227.3	271.7	424.7	160.0	190.2	186.8	257.6
	172	239.3	201.5	249.7	355.8	148.0	171.5	176.0	223.4
	277	213.3	171.8	225.7	344.8	122.7	148.3	164.0	191.8
	365	209.3	121.0	196.0	163.7	106.0	174.0	153.7	170.5
GGT (U/L)	Pre	71.2	53.8	55.5	59.0	46.5	66.7	47.8	61.0
	4	63.0	54.3	51.3	105.2	44.7	60.8	45.0	62.8
	22	74.7	60.3	58.7	79.7	47.8	67.5	49.7	68.3
	88	73.8	65.5	58.0	82.2	49.2	68.0	51.7	67.8
	172	73.3	62.8	59.5	81.5	48.2	68.7	51.5	66.2
	277	65.5	57.3	56.0	79.2	43.3	63.3	45.7	58.8
	365	74.0	49.7	51.0	64.3	43.7	73.0	47.3	68.5
LDH (U/L)	Pre	446.3	425.2	452.7	500.0	324.0	323.8	332.0	331.5
	4	526.2	430.3	448.5	3120.5	476.0	406.0	412.8	420.0
	22	390.2	323.2	343.0	333.5	336.5	324.3	315.8	317.2
	88	536.5	390.0	447.8	473.2	402.8	418.8	370.7	404.4
	172	594.8	364.2	443.0	512.0	437.5	353.2	353.2	401.6
	277	649.7	443.3	495.3	526.8	454.3	446.3	394.5	410.6
	365	429.3	313.3	396.0	480.7	569.3	304.7	378.7	328.0

Gross Pathology and Organ Weights

Reductions in spleen size were observed in 1 MDF and 1 HDF on Day 277 and 1 HDM on Day 365. Decreases in spleen weight were observed in MDM, LDF, MDF, and HDF on Day 277, and LDM, MDM, HDF, and LDF on Day 365.

	Males			Females		
Group	**2**	**3**	**4**	**2**	**3**	**4**
Dose (mg/kg)	**0.3**	**1.0**	**3.0/2.0**	**0.3**	**1.0**	**3.0/2.0**
No. Animals per Group	**3**	**3**	**3**	**3**	**3**	**3**
Spleen (No. Weighed)[a]	3	3	3	3	3	3
Absolute value	1	-1	7	-18	-25	-20
% of body weight	-3	-10	12	-9	-19	-16
% of brain weight	3	-13	-1	-18	-20	-19

[a] All values expressed as percent difference from control group means

(Day 277 spleen weight; Sponsor's Table)

	Males			Females		
Group	**2**	**3**	**4**	**2**	**3**	**4**
Dose (mg/kg)	**0.3**	**1.0**	**3.0/2.0**	**0.3**	**1.0**	**3.0/2.0**
No. Animals per Group	**3**	**3**	**3**	**3**	**3**	**3**
Spleen (No. Weighed)[a]	3	3	3	3	3	3
Absolute value	**-38**	-21	**-45**	-23	-7	10
% of body weight	**-40**	-19	**-42**	-24	-25	4
% of brain weight	**-37**	-21	**-47**	-18	1	11

[a] All values expressed as percent difference from control group means.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group; refer to data tables for actual significance levels and tests used.

(Day 365 spleen weight; Sponsor's Table)

Histopathology

Adequate Battery: Yes

Animal identification	Large intestine, cecum
Artery, aorta	Large intestine, colon
Bone marrow smear	Large intestine, rectum
Bone marrow, femur	Larynx
Bone marrow, sternum	Liver
Bone, femur	Lung
Bone, sternum	Lymph node, mandibular
Brain	Lymph node, mesenteric
Cervix	Small intestine, duodenum
Epididymis[b]	Small intestine, ileum
Esophagus	Small intestine, jejunum
Eye[a]	Muscle, skeletal
Gallbladder	Nerve, optic[a]
Gland, adrenal	Nerve, sciatic
Gland, harderian	Ovary
Gland, mammary gland	Pancreas
Gland, parathyroid	Skin
Gland, pituitary	Spinal cord
Gland, prostate	Spleen
Gland, salivary	Stomach
Gland, seminal vesicle	Testis[b]
Gland, thyroid	Thymus
Gross lesions/masses	Tongue
Gut-associated lymphoid tissue	Trachea
Heart	Urinary bladder
Infusion site	Uterus
Kidney	Vagina

[a] Preserved in Davidson's fixative.
[b] Preserved in Modified Davidson's fixative.

(Sponsor's Table)

Signed Pathology Report: Yes

Peer Review: Yes

Histological Findings: Drug-related histologic findings included hepatocellular vacuolation and single cell necrosis, reactive sinusoidal cells, inflammatory infiltration, and pigment deposits in the liver, and hypocellularity in the spleen. Histological findings generally resolved over the recovery period; however, pigment deposits were observed on Day 365 in 1/3 MDM and 1/3 HDM.

Finding	Males (mg/kg)				Females (mg/kg)			
	0.0	0.3	1	3.0	0.0	0.3	1	3.0
	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3
Liver								
Centrilobular Hepatocyte Vacuolation	**0**	**0**	**3**	**3**	**0**	**0**	**1**	**2**
minimal	0	0	3	0	0	0	1	1
slight	0	0	0	0	0	0	0	1
moderate	0	0	0	2	0	0	0	0
marked	0	0	0	1	0	0	0	0
Single Cell Necrosis	**0**	**0**	**2**	**3**	**0**	**0**	**0**	**3**
minimal	0	0	2	0	0	0	0	3
slight	0	0	0	2	0	0	0	0
moderate	0	0	0	1	0	0	0	0
Reactive Sinusoidal Cells	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**1**
minimal	0	0	0	2	0	0	0	1
Mixed Cell Infiltration	**0**	**0**	**2**	**3**	**0**	**0**	**0**	**1**
minimal	0	0	2	0	0	0	0	1
slight	0	0	0	3	0	0	0	0
Pigment Deposition	**0**	**0**	**1**	**2**	**0**	**0**	**1**	**1**
minimal	0	0	0	0	0	0	1	1
slight	0	0	1	1	0	0	0	0
moderate	0	0	0	1	0	0	0	0

Special Evaluation

Vitamin A, transthyretin (TTR), and T_3 and T_4 Evaluation

Blood samples were collected prior to infusion on Days 1, 22, 127, and 274 and prior to necropsy on Days 277 and 365. There were no drug effects on serum T_3 levels. Drug-related decreases in serum T_4, TTR, and vitamin A levels resolved over the recovery period.

Finding	Day	Males (mg/kg)				Females (mg/kg)			
		0	0.3	1.0	3.0	0	0.3	1.0	3.0
T_4 (ng/mL)	1	4.823	4.140	4.755	5.987	5.483	5.083	4.812	5.147
	22	4.632	3.995	3.602	3.943	4.625	4.048	3.313	2.893
	127	4.313	***2.647****	3.362	3.620	4.912	4.023	3.940	3.504
	274	4.765	***2.893****	***2.878****	***3.132****	5.130	4.622	3.608	3.406
	277	4.607	***2.660****	***2.563****	***2.287****	5.923	4.320	5.053	3.863
	365	4.313	3.750	4.653	5.383	5.320	5.120	4.263	4.715
TTR (μg/mL)	1	328.657	260.218	315.248	307.615	309.442	377.818	361.890	274.438
	22	317.480	82.252	***22.055****	***10.805****	257.857	122.340	***23.105****	***9.405****
	127	283.550	45.397	***12.070****	***7.098****	282.083	99.350	***16.000****	***8.710****
	274	309.992	30.198	***10.993****	***6.770****	275.540	95.475	***16.530****	***8.626****
	277	251.650	12.080	***5.310****	5.337	289.570	58.110	7.303	***5.293****
	365	305.350	180.732	282.783	242.277	238.103	311.937	303.590	231.820
Vitamin A (ng/mL)	1	344.7	267.7	311.2	319.8	335.5	348.2	358.2	342.7
	22	326.3	***88.7****	***66.2****	***60.8****	297.2	157.3	***66.2****	***56.8****
	127	363.5	***99.3****	***96.7****	***77.7****	295.3	170.3	***85.2****	***69.2****
	274	381.8	102.5	***79.3****	***85.7****	340.5	163.3	***99.3****	***84.8****
	277	296.7	***114.3****	***82.3****	***87.3****	385.0	***134.7****	***86.0****	***78.7****
	365	812.0	716.7	534.0	823.0	874.3	684.7	760.3	584.5

Anti-PEG Antibodies
Blood samples were collected prior to infusion on Days 1, 22, 43, 127, and 274 and prior to necropsy on Days 277 and 365. Anti-PEG antibodies were detected in 2 LDF on Day 22. No anti-PEG antibodies were detected after the recovery period.

Complement Activation, and total IgG and IgM
Factor B, total C3, CH50, and C3a were evaluated pretreatment, during Weeks 13, 26, 39, and at the end of recovery. There were no drug effects on factor B, total C3, or CH50. A 2-fold increase in C3a relative to baseline was observed on Day 43 in 4 MDM and 2 HDF.

Cytokine Stimulation
Circulating IL-1β, IL-1RA, IL-6, IFN-γ, and TNF-α were evaluated prior to and 3 h after dosing on Day 43. There were no drug effects on IL-1β, IFN-γ, TNF-α, or IFN-α. IL-1RA levels were generally comparable between control and active groups; however, a 27.4-fold increase in 1 HDM was thought by the sponsor to be possibly drug related. Increases in IL-6 levels were also similar between control and active groups, although the sponsor considered the increases in 1 HDM and 1 HDF to be possibly potentiated by ALN-TTR02.

Male Reproductive Assessments
Reproductive assessments were conducted at the end of the dosing period. There were no drug effects on spermatogenic cycle, sperm parameters, or testis length or volume.

Toxicokinetics

Increases in plasma C_{max} and AUC on Day 1 for ALN-18328 and PEG_{2000}-C-DMG were greater than dose proportional and dose proportional for DLin-MC3-DMA. Increases in C_{max} and AUC on Day 274 for ALN-18328, PEG_{2000}-C-DMG, and DLin-MC3-DMA dose proportional.

Parameter	Sex	Day 1 (mg/kg)			Day 274 (mg/kg)		
		0.3	1.0	3.0/2.0	0.3	1.0	3.0/2.0
ALN-18328							
$t_{1/2}$ (h)	M	11.2	8.98	13.5	12.7	17.4	19.4
	F	15	7.88	20.1	--	13.3	14.8
C_{max} (μg/mL)	M	3.54	10.4	43.1	4.63	12.3	35.8
	F	3.15	10.0	50.6	4.52	14.3	34.5
AUC_{inf} (μg × h/mL)	M	13.1	26.0	134	23.9	57.9	89.5
	F	11.2	23.5	218	--	58.0	128
DLin-MC3-DMA							
C_{max} (μg/mL)	M	33.5	86.3	265	34.9	112	330
	F	30.0	87.2	277	41.2	129	318
AUC_{inf} (μg × h/mL)	M	683	3350	4750	2590	6040	8730
	F	1030	2080	5290	1580	5520	7460
PEG_{2000}-C-DMG							
C_{max} (μg/mL)	M	3.66	13.1	53.6	4.47	15.8	33.6
	F	3.37	12.1	50.3	4.21	15.7	32.4
AUC_{inf} (μg × h/mL)	M	64.2	276	955	84.6	339	722
	F	60.0	243	632	73.2	313	552

Dosing Solution Analysis

Dosing solutions were within 15% of their respective target concentrations.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: ALN-TTR02: Bacterial Reverse Mutation Assay

Study no.: TTR02-NCD10-015
Study report location: EDR
Conducting laboratory and location: (b) (4) [redacted]
Date of study initiation: January 7, 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: ALN-TTR02, Lot L00096, 87.7%

Methods

Strains: TA98, TA100, TA1535, TA1537, and WP2 *uvr*A
Concentrations in definitive study: 0, 50, 150, 500, 1500, 5000 μg/plate
Basis of concentration selection: Preliminary study
Negative control: PBS
Positive control:

Strain	-S9	+S9
TA98	2-nitrofluorene	2-aminoanthracine
TA100	sodium azide	2-aminoanthracine
TA1535	sodium azide	2-aminoanthracine
TA1537	9-aminoacridine	2-aminoanthracine
WP2*uvrA*	methyl methanesulfonate	2-aminoanthracine

Formulation/Vehicle: PBS
Incubation & sampling time: 48 to 72 h

Results

ALN-TTR02 was negative in an OECD-compliant Ames assay.

Study title: DLin-MC3-DMA: Bacterial Reverse Mutation Assay

Study no.: PSC02-NCD10-006
Study report location: EDR
Conducting laboratory and location: (b) (4) [redacted]
Date of study initiation: November 12, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: DLin-MC3-DMA, Lot WO688, 98.4%

Methods

Strains: TA98, TA100, TA1535, TA1537, and WP2 *uvr*A
Concentrations in definitive study: 0, 50, 150, 500, 1500, 5000 μg/plate
Basis of concentration selection: Preliminary study
Negative control: EtOH
Positive control:

Strain	-S9	+S9
TA98	2-nitrofluorene	2-aminoanthracine
TA100	sodium azide	2-aminoanthracine
TA1535	sodium azide	2-aminoanthracine
TA1537	9-aminoacridine	2-aminoanthracine
WP2*uvr*A	methyl methanesulfonate	2-aminoanthracine

Formulation/Vehicle: EtOH
Incubation & sampling time: 48 to 72 h

Results

DLin-MC3-DMA was negative in an OECD-compliant Ames assay.

Study title: PEG2000-C-DMG: Bacterial Reverse Mutation Assay

Study no.: TTR02-NCD10-009
Study report location: EDR
Conducting laboratory and location: (b) (4) [redacted]

Date of study initiation: November 11, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: PEG2000-C-DMG, Lot G08202, 100%

Methods

Strains: TA98, TA100, TA1535, TA1537, and WP2 *uvr*A
Concentrations in definitive study: 0, 50, 150, 500, 1500, 5000 μg/plate
Basis of concentration selection: Preliminary study
Negative control: DMSO
Positive control:

Strain	-S9	+S9
TA98	2-nitrofluorene	2-aminoanthracine
TA100	sodium azide	2-aminoanthracine
TA1535	sodium azide	2-aminoanthracine
TA1537	9-aminoacridine	2-aminoanthracine
WP2*uvr*A	methyl methanesulfonate	2-aminoanthracine

Formulation/Vehicle: DMSO
Incubation & sampling time: 48 to 72 h

Results

PEG$_{2000}$-C-DMG was negative in an OECD-compliant Ames assay.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: ALN-TTR02: *In Vitro* Mammalian Chromosome Aberration Test

Study no.:	TTR-NCD10-016
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	January 5, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALN-TTR02, L00096, 87.7%

Methods

Cell line:	Human peripheral blood lymphocytes
Concentrations in definitive study:	-S9 (4 h):313, 625, 1250, 2500, 5000 μg/mL -S9 (20 h):40, 78, 156, 313, 500, 625 μg/mL +S9(4h): 313, 625, 1250, 2500, 5000 μg/mL
Basis of concentration selection:	50% reduction in mitotic index at the high dose
Negative control:	PBS
Positive control:	Mitomycin C (-S9), cyclophosphamide (+S9)
Formulation/Vehicle:	PBS
Incubation & sampling time:	4 and 20 h incubation (-S9), 4 h incubation (+S9). Cells were harvested 20 h after initiation of dosing.

Results

ALN-TTR02 was negative in an OECD-compliant *in vitro* mammalian chromosome aberration assay.

Study title: DLin-MC3-DMA: *In Vitro* Mammalian Chromosome Aberration Test

Study no.:	PCS02-NCD10-007
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	November 9, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	DLin-MC3-DMA, Lot W0688, 98.4%

Methods

Cell line:	Human peripheral blood lymphocytes
Concentrations in definitive study:	-S9 (4 h): 0, 25, 50, 100 μg/mL -S9 (20 h): 0, 25, 50, 100 μg/mL +S9 (4h): 0, 25, 50, 2000 μg/mL
Basis of concentration selection:	Precipitation
Negative control:	EtOH
Positive control:	Mitomycin C (-S9), cyclophosphamide (+S9)
Formulation/Vehicle:	EtOH
Incubation & sampling time:	4 and 20 h incubation (-S9), 4 h incubation (+S9). Cells were harvested 20 h after initiation of dosing.

Results

DLin-MC3-DMA was negative in an OECD-compliant *in vitro* mammalian chromosome aberration assay.

Study title: PEG2000-C-DMG: *In Vitro* Mammalian Chromosome Aberration Test

Study no.:	TTR02-NCD10-008
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	November 9, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PEG2000-C-DMG, G08202, 100%

Methods

Cell line:	Human peripheral blood lymphocytes

Concentrations in definitive study:	-S9 (4 h): 0, 25, 50, 150 μg/mL -S9 (20 h): 0, 25, 50, 100 μg/mL +S9(4h): 0, 12.5, 50, 150 μg/mL
Basis of concentration selection:	50% reduction in mitotic index at the high dose
Negative control:	Water
Positive control:	Mitomycin C (-S9), cyclophosphamide (+S9)
Formulation/Vehicle:	Water
Incubation & sampling time:	4 and 20 h incubation (-S9), 4 h incubation (+S9). Cells were harvested 20 h after initiation of dosing.

Results

PEG_{2000}-C-DMG was negative in an OECD-compliant *in vitro* mammalian chromosome aberration assay.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Mammalian Erythrocyte Micronucleus Test in Mouse Bone Marrow

Study no:	TTR02-NCD12-006
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	91.7%
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALN-TTR02, Lot L00114,

Methods

Doses in definitive study:	0, 7.5, 15, 30 mg/kg
Frequency of dosing:	Single dose
Route of administration:	IV injection
Dose volume:	20 mL/kg
Formulation/Vehicle:	Saline
Species/Strain:	CD1 mice
Number/Sex/Group:	5/sex/group
Satellite groups:	None
Basis of dose selection:	Dose range finding study (20, 30, 40 mg/kg)
Negative control:	Saline
Positive control:	Mitomycin C

Results

ALN-TTR02 was negative in an OECD-compliant mammalian erythrocyte micronucleus assay in mice.

8 Carcinogenicity

Dose Ranging Studies (Summarized from a review by D. Hawver under IND 117395)
A 26-week dose-ranging study in C57BL/6 mice was conducted but was not appropriate for dose selection in TgRasH2 mice. An additional range-finding study was conducted in wild type and TgRasH2 mice, based on which doses of 0, 0.5, 3, and 6 mg/kg ALN-TTR02 were recommended by the Executive CAC for evaluation in a 6-month carcinogenicity study in TgRasH2 mice.

Study title: Patisiran (ALN-TTR02): A 26-week Intravenous Injection Carcinogenicity Study in TgRasH2 Mice

Study no.:	TTR02-GLP15-024
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	October 13 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALN-TTR02, Batch B140288, 93%
Executive CAC concurrence:	Yes

Methods

Doses:	0, 0.5, 2, 6 mg/kg (ALN-TTR02); 75 mg/kg (MNU)
Frequency of dosing:	Once every two weeks (ALN-TTR02); Single dose (MNU)
Dose volume:	10 mL/kg
Route of administration:	IV injection
Formulation/Vehicle:	Saline
Basis of dose selection:	Doses were recommended by the Executive CAC based on a 23% reduction in body weight gain in males and moderately increased bone marrow myeloid cellularity in females at 6 mg/kg in a 2-month range-finding study.
Species/Strain:	TgRasH2 mice
Number/Sex/Group:	25/sex/group
Age:	9 weeks at initiation of dosing
Animal housing:	Animals were single housed. Harlan Diet #2016C was provided *ad libitum* throughout the study.
Paradigm for dietary restriction:	None
Dual control employed:	No
Interim sacrifice:	None
Satellite groups:	TK; 12/sex/group (control); 47/sex/group (ALN-TTR02) ADA; 3/sex/group
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality

Animals were monitored twice daily for mortality or signs of morbidity. There were no drug-related effects on survival. However, significant mortality was observed in the positive control groups.

Text Table 11
Summary of Survival and Noteworthy Cause of Death

	Male					Female				
Group Dose (mg/kg)	1 0[a]	2 0.5	3 2	4 6	5 75[b]	1 0[a]	2 0.5	3 2	4 6	5 75[b]
Survivability	25/25	25/25	22/25	23/25	10/25	22/25	23/25	23/25	23/25	4/25
Survival rate	100%	100%	88%	92%	40%	88%	92%	92%	92%	16%
Cause of Death										
Lymphoma	0	0	0	0	14	1	0	0	0	20
Hemangiosarcoma	0	0	1	1	0	0	0	1	1	0
Squamous cell carcinoma	0	0	0	0	0	1	1	0	0	1
Accidental	0	0	0	0	0	0	1	0	0	0
Undetermined	0	0	0	0	1	1	0	0	1	0

[a] Saline
[b] N-Methyl-N-Nitrosourea (MNU); positive control

(Sponsor's Table)

Clinical Signs and Palpable Masses

Detailed clinical observations were conducted weekly; there were no drug-related clinical signs. Palpable masses were found in 1/25 HDM (inguinal) and 1/25 HDF (sacral).

Text Table 12
Incidence (Number of Animals) of Clinically Observed Masses

	Males					Females				
Group Dose (mg/kg)	1 0[a]	2 0.5	3 2	4 6	5 75[b]	1 0[a]	2 0.5	3 2	4 6	5 75[b]
Number of mice	25	25	25	25	25	25	25	25	25	25
Number of mice with masses	0	0	0	1	5	0	0	0	1	4
Percent of mice with masses (%)	0	0	0	4	20	0	0	0	4	16

[a] Saline
[b] N-Methyl-N-Nitrosourea (MNU); positive control

(Sponsor's Table)

Body Weights

Animals were weighed weekly. Drug-related reductions in BW of 3 to 6% relative to control were observed in males; there were no drug-related effects on BW in females.

Group	Body Weight (g)		Gain (g)	% of control gain	% relative change in BW
	Week 1	Week 26			
Males					
Saline	24	33	9	--	--
0.5 mg/kg - Low	24	32	8	89	-3
2 mg/kg - Mid	24	32	8	89	-3
6 mg/kg - High	24	31	7	78	-6
MNU	24	29	5	56	-12
Females					
Saline	19	23	4	--	--
0.5 mg/kg - Low	19	23	4	100	0
2 mg/kg - Mid	18	24	6	150	4
6 mg/kg - High	18	23	5	125	0
MNU	19	24	5	125	4

Food Consumption

Food consumption was recorded weekly. There were no ALN-TTR02 related effects on food consumption.

Hematology

Blood samples were collected from main study animals at scheduled necropsy. There were no ALN-TTR02 related effects on hematology.

Gross Pathology

There were no ALN-TTR02 related gross pathology findings.

Histopathology

Signed Pathology Report: Yes
Peer Review: Yes
Non-neoplastic findings: Single cell hepatocyte necrosis was observed in 15/25 HDM.
Neoplastic findings: According to the sponsor, there were no statistically significant increases in tumor incidence. Bronchioalveolar adenomas were observed at all doses of ALN-TTR02 in males, but were not present in the saline control or in females. Bronchioalveolar carcinomas were observed in 1 MDM and 1 CF. There was a possible dose-related increase in the frequency of bronchioalveolar adenoma in males. Based on historical data provided by the sponsor, the frequency of bronchioalveolar adenomas and carcinomas exceeded 1%, indicating that such findings are common in TgRas mice. An analysis and review by the CDER Division of Biometrics concluded that there were no statistically-significant increases in tumors.

Dose ALN-TTR02 (mg/kg)	Males				Females			
	0	0.5	2	6	0	0.5	2	6
No. Animals Examined	25	25	25	25	25	25	25	25
Bronchioalveolar adenoma	0	2	2	3	0	0	0	0
Bronchioalveolar carcinoma	0	0	2	0	1	0	0	0

Neoplastic findings in lung

Year	2010	2013	2014
No. Animals Examined	50	50	25
Bronchioalveolar adenoma (lung)	6	1	1
Bronchioalveolar carcinoma (lung)	1	1	0

Historical range for CRL Montreal ULC

Toxicokinetics

Plasma TK for the siRNA moiety (ALN-18328) were evaluated on Day 183; blood samples were collected from satellite animals. Increases in plasma C_{max} and AUC for ALN-18328 were generally dose-proportional. There were no sex differences in TK parameters.

ALN-18328									
	Male			Female			Ratio (F/M) [b]		
siRNA (mg/kg)[a]	0.5	2	6	0.5	2	6	0.5	2	6
$t_{1/2}$ (h)	1.1	3.3	6.0	0.52	1.4	5.7	NA	NA	NA
t_{max} (h)	0.083	0.083	0.50	0.083	0.083	0.083	NA	NA	NA
C_{max} (μg/mL)	7.90	32.4	79.6	7.05	31.0	81.1	0.892	0.957	1.02
AUC_{0-t} (μg·h/mL)	12.6	54.8	209	9.68	32.6	183	0.768	0.595	0.875
C_{max}/Dose	15.8	16.2	13.3	14.1	15.5	13.5	0.892	0.957	1.02
AUC_{0-t}/Dose	25.2	27.4	34.8	19.4	16.3	30.5	0.768	0.595	0.875
CL (mL/h/kg)	39.3	36.4	27.6	51.5	60.6	31.9	NA	NA	NA
V_d (mL/kg)	42.9	108	137	37.5	73.8	125	NA	NA	NA

(Sponsor's Table)

Dosing Solution Analysis

Dosing solutions were within 15% of their respective target concentrations.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Patisiran (ALN-TTR02) and AF-011-18534: An Intravenous Dose Range-Finding Embryo-Fetal Development Study in Sprague Dawley Rats

Study no.:	TTR02-DSM15-015
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	April 2, 2015
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	ALN-TTR02, Lot B140288, 93% AF-011-18534, Lot 856134, 86%

Findings

Female rats were administered 0, 0.15, 0.5, or 1.5 mg/kg AF-011-18534 (mouse/rat surrogate) or 1.5 mg/kg ALN-TTR02 15, 8, and 1 day prior to mating with non-dosed males, and on GD 6, 13, and 19. Main study animals were euthanized on GD 21. All rats survived until scheduled necropsy; there were no drug-related clinical signs. AF-011-18534 did not affect body weights, food consumption, or clinical chemistry; however, ALN-TTR02 administration resulted in reductions in body weight of 44% relative to controls and elevations of 87 and 51% in mean ALT and AST, respectively. There were no effects of AF-011-18534 or ALN-TTR02 on mating and fertility, ovarian or uterine parameters, or fetal parameters. There was no fetal transfer of siRNA or PEG_{2000}-C-DMG, but approximately 0.4% transfer of DLin-MC3-DMA.

Study title: ALN-TTR02: An Intravenous Dosage Range-finding Development Toxicity Study in Rats Including a Toxicokinetic Evaluation

Study no.:	TTR02-NCD13-003
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	February 19, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALN-TTR02, Lot L00114, 91.7% AF-011-18534, Lot 902031, 96.1

Methods

Doses:	0, 0.03, 0.1, 0.3, or 1 mg/kg ALN-TTR02; 0.3 or 1 mg/kg AF-011-18534 (mouse/rat surrogate)
Frequency of dosing:	See Study Design
Dose volume:	12 mL/kg
Route of administration:	IV infusion (1 h)
Formulation/Vehicle:	Saline
Species/Strain:	SD rats
Number/Sex/Group:	8/group
Satellite groups:	TK arm (15/group)
Study design:	Female rats were dosed 3 days prior to mating, and on GD 6 and 13. Cesarean sections were performed on GD 21.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality and Clinical Signs

Rats were evaluated twice daily for mortality or signs of morbidity. There were no drug-related mortalities or clinical signs.

Body Weight and Food Consumption

Body weights were recorded daily during the dosing phase. Food consumption was recorded weekly. There were no drug effects on body weights or food consumption.

Hematology and Clinical Chemistry

Blood samples from main study animals were collected approximately 24 h after the final dose; there were no drug effects.

Vitamin A, T_3, and T_4

Administration of ALN-TTR02 did not affect vitamin A, T_3, or T_4 levels. Administration of AF-011-18534 resulted in 4- to 10-fold decreases in mean vitamin A levels and 67 to 76% reductions in mean T_4 levels.

Toxicokinetics

Increases in plasma C_{max} for ALN-18328 were slightly greater than dose-proportional. Increases in C_{max} and AUC for DLin-MC3-DMA and PEG_{2000}-C-DMG were greater than dose proportional.

ALN-18328

	Day 1				Day 13				R			
siRNA (mg/kg)	0.03	0.1	0.3	1	0.03	0.1	0.3	1	0.03	0.1	0.3	1
Tmax (h)	1.083	1.083	1.083	1.083	1.083	1.083	1.083	1.083	NA	NA	NA	NA
Cmax (ng/mL)	182	651	2303	7830	80	801	2009	2525	0.44	1.23	0.87	0.32
AUC_{0-t} (h·ng/mL)	NR	NR	NR	9877	NR	1159	NR	NR	NA	NA	NA	NA

Tmax and AUC values based on time from start of infusion (SOI).
NR = not reportable, does not meet acceptance criteria for reporting AUC (4 consecutive quantifiable time points)

DLin-MC3-DMA

	Day 1				Gestation Day 13				R			
siRNA (mg/kg)	0.03	0.1	0.3	1	0.03	0.1	0.3	1	0.03	0.1	0.3	1
Lipid (mg/kg)	0.22	0.72	2.17	7.23	0.22	0.72	2.17	7.23	0.22	0.72	2.17	7.23
Tmax (h)	1.083	1.083	1.083	1.083	1.083	1.083	1.083	1.083	NA	NA	NA	NA
Cmax (ng/mL)	1286	6800	13767	65033	562	6300	13797	16250	0.44	0.93	1.00	0.25
AUC_{0-t} (h·ng/mL)	2771	13401	28392	117296	2157	15718	43515	83844	0.78	1.17	1.53	0.71
AUC_{0-73} (h·ng/mL)	2771	13401	28392	117296	1419	12654	33831	50076	0.51	0.94	1.19	0.43

Tmax and AUC values based on time from start of infusion (SOI).

PEG_{2000}-C-DMG

	Day 1				Gestation Day 13				R			
siRNA (mg/kg)	0.03	0.1	0.3	1	0.03	0.1	0.3	1	0.03	0.1	0.3	1
Lipid (mg/kg)	0.03	0.09	0.26	0.85	0.03	0.09	0.26	0.85	0.03	0.09	0.26	0.85
Tmax (h)	1.083	1.083	1.083	1.083	1.083	1.083	1.083	1.083	NA	NA	NA	NA
Cmax (ng/mL)	161	514	1777	6747	84	776	1735	3265	0.52	1.51	0.98	0.48
AUC_{0-t} (h·ng/mL)	299	1433	4600	17857	165	2141	4307	9469	0.55	1.49	0.94	0.53
AUC_{0-73} (h·ng/mL)	336	1658	4600	17857	201	1694	4408	8846	0.60	1.02	0.96	0.50

Tmax and AUC values based on time from start of infusion (SOI).

(Sponsor's Tables)

Dosing Solution Analysis

Dosing solutions were within 15% of their respective target concentrations.

Necropsy

There were no drug effects on fertility index, corpora lutea, pre- or postimplantation loss, litter size, or offspring weight.

Study title: Patisiran (ALN-TTR02) and AF-011-18534: An Intravenous Fertility and General Reproduction Study in Male Sprague Dawley Rats

Study no.: TTR02-GLP15-035
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: November 24, 2015
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: ALN-TTR02, Lot B140288, 93%

AF-011-18534, Lot 856134, 86%

Methods

Doses:	0, 0.03, 0.1, or 0.3 mg/kg ALN-TTR02; 0.1 mg/kg AF-011-18534
Frequency of dosing:	See study design
Dose volume:	12 mL/kg
Route of administration:	IV infusion (1 h)
Formulation/Vehicle:	Saline
Species/Strain:	SD rats
Number/Sex/Group:	24/group
Satellite groups:	TK (9/group)
Study design:	Male SD rats were administered test article every other week, beginning 10 weeks prior to mating.
Deviation from study protocol:	No significant deviations.

Observations and Results

Mortality and Clinical Signs

Animals were observed twice daily for mortality or signs of morbidity. There were no drug-related mortalities or clinical signs.

Body Weight and Food Consumption

Body weights were recorded daily during the dosing phase. Food consumption was recorded weekly. There were no drug effects on body weights or food consumption.

Toxicokinetics

Increases in plasma C_{max} and AUC for ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG were generally dose-proportional.

ALN-18328									
	Day 1			Day 85			Ratio[c]		
Patisiran (mg/kg)[a]	**0.03**	**0.1**	**0.3**	**0.03**	**0.1**	**0.3**	**0.03**	**0.1**	**0.3**
$t_{1/2}$ (h)[b]	ND	ND	1.57	ND	1.89	ND	NA	NA	NA
t_{max} (h)[b]	1.1	2.0	1.1	1.1	1.1	1.1	NA	NA	NA
C_{max} (μg/mL)	0.309	1.18	1.86	0.320	1.03	1.92	1.04	0.870	1.03
AUC_{last} (μg·h/mL)[b]	0.424	3.35	4.63	0.459	2.18	3.48	1.08	0.651	0.750
C_{max}/Dose	10.3	11.8	6.21	10.7	10.3	6.40	1.04	0.870	1.03
AUC_{last}/Dose	14.1	33.5	15.4	15.3	21.8	11.6	1.08	0.651	0.750
CL (mL/h/kg)[b]	ND	ND	63.6	ND	44.4	ND	NA	NA	NA
V_d (mL/kg)[b]	ND	ND	118	ND	86.8	ND	NA	NA	NA
DLin-MC3-DMA									
	Day 1			Day 85			Ratio[c]		
Patisiran (mg/kg)[a]	**0.03**	**0.1**	**0.3**	**0.03**	**0.1**	**0.3**	**0.03**	**0.1**	**0.3**
$t_{1/2}$ (h)[b]	78.9	ND	125	ND	ND	389	NA	NA	NA
t_{max} (h)[b]	1.1	1.1	1.1	1.1	1.1	1.1	NA	NA	NA
C_{max} (μg/mL)	1.26	6.07	22.6	2.63	6.33	12.2	2.09	1.04	0.540
AUC_{last} (μg·h/mL)[b]	8.70	20.8	82.7	12.7	18.1	107	1.46	0.874	1.29
C_{max}/Dose	6.47	9.37	11.6	13.5	9.77	6.28	2.09	1.04	0.540
AUC_{last}/Dose	44.8	32.1	42.6	65.5	28.0	55.0	1.46	0.874	1.29
CL (mL/h/kg)[b]	21.8	ND	21.8	ND	ND	17.7	NA	NA	NA
V_d (mL/kg)[b]	1000	ND	1730	ND	ND	3000	NA	NA	NA
PEG_{2000}-C-DMG									
	Day 1			Day 85			Ratio[c]		
Patisiran (mg/kg)[a]	**0.03**	**0.1**	**0.3**	**0.03**	**0.1**	**0.3**	**0.03**	**0.1**	**0.3**
$t_{1/2}$ (h)[b]	ND	ND	ND	ND	ND	4.39	NA	NA	NA
t_{max} (h)[b]	2.0	2.0	2.0	2.0	2.0	2.0	NA	NA	NA
C_{max} (μg/mL)	0.0878	0.225	1.14	0.162	0.464	1.08	1.84	2.06	0.950
AUC_{last} (μg·h/mL)[b]	ND	ND	3.55	ND	ND	7.29	NA	NA	2.05
C_{max}/Dose	3.84	2.95	4.97	7.07	6.09	4.72	1.84	2.06	0.950
AUC_{last}/Dose	ND	ND	15.5	ND	ND	31.9	NA	NA	2.05
CL (mL/h/kg)[b]	ND	ND	ND	ND	ND	30.7	NA	NA	NA
V_d (mL/kg)[b]	ND	ND	ND	ND	ND	158	NA	NA	NA

NA: not applicable; ND: not determined.
Many samples analyzed for PEG_{2000}-C-DMG concentrations after patisiran dosing had no quantifiable results due to insufficient sample volume
[a] siRNA doses of 0.03, 0.1 and 0.3 mg/kg correspond to DLin-MC3-DMA doses of 0.19, 0.65 and 1.94 mg/kg, respectively, which were used to calculate the TK parameters for DLin-MC3-DMA, and corresponded to PEG_{2000}-C-DMG doses of 0.02, 0.08 and 0.23 mg/kg, respectively, which were used to calculate the TK parameters for PEG_{2000}-C-DMG.
[b] Time from start of infusion (SOI) was used to calculate the parameter
[c] Ratio = Day 85 TK parameter/Day 1 TK parameter

(Sponsor's Table)

Hematology and Clinical Chemistry

Blood samples were collected at necropsy from fasted animals. There were no drug effects on hematology or clinical chemistry parameters. Administration of ALN-TTR02 did not affect vitamin A, T_3, or T_4 levels; however, administration of the mouse/rat surrogate (AF-011-18534) reduced vitamin A and T_4 levels.

Male Vitamin A Levels (µg/mL)			
	Number Tested	Mean	Standard Deviation
0 (Control) mg/kg	10	0.210	0.018
0.03 mg/kg (patisiran)	10	0.209	0.035
0.1 mg/kg (patisiran)	10	0.237	0.039
0.3 mg/kg (patisiran)	10	0.242	0.058
0.1 mg/kg (AF-011-18534)	10	0.045	0.007

		T3 Concentration (ng/mL)		T4 Concentration (µg/dL)	
	Number Tested	Mean	Standard Deviation	Mean	Standard Deviation
0 (Control) mg/kg	10	1.10	0.16	4.34	0.56
0.03 mg/kg (patisiran)	10	1.12	0.19	3.53	0.59
0.1 mg/kg (patisiran)	10	1.15	0.21	3.69	0.45
0.3 mg/kg (patisiran)	10	0.95	0.32	3.47	0.60
0.1 mg/kg (AF-011-18534)	10	0.85	0.10	1.39a	0.32a

[a] – 7 of 10 males below lower level of quantification (1.00 ng/dL). Mean and standard deviation based on N=3.

(Sponsor's Tables)

Dosing Solution Analysis

Dosing solutions were within 15% or their respective target concentrations.

Necropsy

There were no drug effects on mating, or sperm parameters or morphology.

Study title: Patisiran (ALN-TTR02) and AF-011-18534: An Intravenous Fertility and Embryo-Fetal Development Study in Sprague Dawley Female Rats

Study no.:	TTR02-GLP15-031
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	October 6, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALN-TTR02, Lot L0409159, 92% AF-011-18534, Lot 856134, 86%

Methods

Doses:	0, 0.15, 0.5, 1.5 mg/kg (ALN-TTR02); 1.5 mg/kg (AF-011-18534)
Frequency of dosing:	See study design
Dose volume:	12 mL/kg
Route of administration:	IV infusion (1 h)
Formulation/Vehicle:	Saline
Species/Strain:	Female SD rats
Number/Sex/Group:	24/group
Satellite groups:	TK (9/group)
Study design:	Test article was administered 15, 8, and 1 day prior to mating, and on GD 6, 13, and 19. Main study and TK animals were euthanized on GD 21 and 20, respectively.
+Deviation from study protocol:	No significant deviations

Observations and Results

Mortality and Clinical Signs+

Animals were observed twice daily for mortality or signs of morbidity. There were no test article-related mortalities or clinical signs.

Body Weight and Food Consumption

Body weights were recorded daily during the dosing phase. Food consumption was recorded weekly during the dosing period. There were no test article-related effects on body weight or food consumption.

Toxicokinetics

Increases in plasma C_{max} and AUC for ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG were greater than dose proportional.

Patisiran Dosing - Day of Gestation 19									
	ALN-18328			Dlin-MC3-DMA			PEG_{2000}-C-DMG		
siRNA (mg/kg)[a]	**0.15**	**0.5**	**1.5**	**0.15**	**0.5**	**1.5**	**0.15**	**0.5**	**1.5**
C_{max} (µg/mL)	1.99	6.28	29.0	12.5	31.5	133	1.63	4.22	17.8
t_{max} (h)[b]	1.08	1.08	1.08	1.08	1.08	1.08	1.08	1.08	1.08
t_{last} (h)[b]	9.0	9.0	25	25	25	25	25	25	25
AUC_{last} (µg.h/mL)[b]	4.62	16.0	88.0	30.1	99.1	361	3.98	11.6	41.1
AUC_{24h} (µg.h/mL)[b]	4.62	16.0	88.0	30.1	99.1	361	3.98	11.6	41.1
AF-011-18534 Dosing - Day of Gestation 19									
	AD-18534			Dlin-MC3-DMA			PEG_{2000}-C-DMG		
siRNA (mg/kg)[c]			**1.5**			**1.5**			**1.5**
C_{max} (µg/mL)			3.98			91.3			19.4
t_{max} (h)[b]			2.0			1.08			1.08
t_{last} (h)[b]			9.0			25			25
AUC_{last} (µg.h/mL)[b]			16.6			348			55.5
AUC_{24h} (µg.h/mL)[b]			16.6			348			55.5

[a] siRNA doses of 0.15, 0.5 and 1.5 mg/kg correspond to DLin-MC3-DMA doses of 0.97, 3.24 and 9.72 mg/kg, respectively, which were used to calculate the TK parameters for DLin-MC3-DMA, and corresponded to PEG_{2000}-C-DMG doses of 0.11, 0.38 and 1.14 mg/kg, respectively, which were used to calculate the TK parameters for PEG_{2000}-C-DMG.
[b] The times from start of infusion (SOI) was used to calculate the parameters.
[c] siRNA doses of 1.5 mg/kg correspond to a DLin-MC3-DMA dose of 10.14 mg/kg, which were used to calculate the TK parameters for DLin-MC3-DMA, and corresponded to a PEG_{2000}-C-DMG dose of 1.17 mg/kg, which were used to calculate the TK parameters for PEG_{2000}-C-DMG.

(Sponsor's Table)

Hematology and Clinical Chemistry

Blood samples were collected at scheduled necropsy from fasted animals. There were no drug effects on hematology parameters. ALN-TTR02 effects on clinical chemistry parameters in HDF included approximately 2-fold elevations in AST and ALT, and reductions of 29, 13, and 19% in total bilirubin, total protein, and albumin, respectively. Vitamin A was reduced by 6, 11, and 22% in LDF, MDF, and HDF, respectively. AF-011-18534 reduced TTR and vitamin A levels by 95 and 88%, respectively. Serum T_3 levels were reduced by 10 % in HDF and 20% by AF-011-18534. There were no test article effects on serum T_4.

Dosing Solution Analysis

All doses were within 15% of their respective target concentrations.

Necropsy

There were no test article-related effects on gestation period, estrous cycle, mating, or pregnancy, or effects on corpora lutea, implantations, preimplantation loss, litter size, or live/dead fetuses. There were no test article-induced external, visceral, or skeletal malformations.

9.2 Embryofetal Development

Study title: Patisiran (ALN-TTR02) and AF-011-18534: A Dose Range-finding Embryo-Fetal Development Study by Intravenous Infusion in Rabbits, including Preliminary Evaluation in Non-Pregnant Rabbits.

Study no.:	TTR02-DSM15-012
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	ALN-TTR02, Batch B140288, 93% AF-011-18534, Batch 856134, 86%

Key Study Findings

Part A

Non-pregnant NZW rabbits (3/group) were administered 0, 0.3, 1, or 3 mg/kg AF-011-18534 or 2 mg/kg ALN-TTR02 by 1 h IV infusion on Days 1, 8, and 15. Drug-related effects included increases in AST and ALT of 51- and 27-fold, respectively, following administration of 3 mg/kg AF-011018534, and increases of 7 and 6-fold in AST and ALT, respectively, following administration of HD ALN-TTR02.

Part B

Pregnant NZW rabbits (5/group) were administered 0, 0.3, 1, or 2 mg/kg ALN-TTR02 by 1 h IV infusion on GD 7, 13, and 19. Main study and TK animals were euthanized on GD 29 and 20, respectively. 1/5 and 3/5 MDF and HDF aborted between GD 21 and 27 and were euthanized. Drug-related clinical signs in MDF and HDF included red material in the cage pan (secondary to abortion) and decreased/absent/liquid feces. Weight loss from GD 7 to 20 of 26 and 92% was observed in MDF and HDF and was accompanied by reductions in food consumption. AST was elevated by 2.7 and 16.8-fold in MDF and HDF, respectively. ALT was increased 8.2-fold in HDF. There were no abnormal findings in animals that survived until scheduled necropsy. Two and 4 early resorptions/litter at MDF and HDF, respectively, were observed following cesarean section. Postimplantation loss was 24 and 50% in MDF and HDF, respectively (control was 13%). Mean litter size was 9.8 (control), 8.8 (MDF), and 4 (HDF) fetuses per litter. There were no drug-related external malformations. TK analysis revealed greater than dose-proportional increases in C_{max} and AUC for ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG. No significant fetal uptake of ALN-18328, DLin-MC3-DMA or PEG_{2000}-C-DMG was observed.

Mean (±SD) Maternal Liver ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG Concentrations (ng/g) on DG 20 (Necropsy) in New Zealand White Rabbits After IV Infusions of Patisiran on DG 7, 13 and 19

Group	Patisiran Dose (mg/kg)	Concentration (ng/g)					
		ALN-18328		DLin-MC3-DMA		PEG_{2000}-C-DMG	
		Mean	SD	Mean	SD	Mean	SD
7	0.3	106	12.1	172,000	62,200	602	247
8	1	454	223	359,000	145,000	808	57.5
9	2	1390	384	689,000	110,000	2390	830

Text Table 18

Mean (±SD) Maternal Kidney ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG Concentrations (ng/g) on DG 20 (Necropsy) in New Zealand White Rabbits After IV Infusions of Patisiran on DG 7, 13 and 19

Group	Patisiran Dose (mg/kg)	Concentration (ng/g)					
		ALN-18328		DLin-MC3-DMA		PEG2000-C-DMG	
		Mean	SD	Mean	SD	Mean	SD
7	0.3	NR	NR	191	25.6	162	18.2
8	1	40.6[a]	NR	1170	369	880	143
9	2	116[b]	NR[c]	2430	410	1640	110

Abbreviations: DG=day of presumed gestation; LLOQ=lower limit of quantitation; NR=not reported – insufficient values to calculate parameter or all concentration values <LLOQ; SD=standard deviation

[a]Concentration value represents n =1

[b]Concentration value represents n=2

Text Table 19

Mean (±SD) Maternal Spleen ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG Concentrations (ng/g) on DG 20 (Necropsy) in New Zealand White Rabbits After IV Infusions of Patisiran on DG 7, 13 and 19

Group	Patisiran Dose (mg/kg)	Concentration (ng/g)					
		ALN-18328		DLin-MC3-DMA		PEG_{2000}-C-DMG	
		Mean	SD	Mean	SD	Mean	SD
7	0.3	236	175	65,800	37,800	1060	406
8	1	141	50.6	138,000	32,700	3170	1010
9	2	910	1070	269,000	97,100	12,400	5650

Abbreviations: DG=day of presumed gestation; SD=standard deviation

(Sponsor's Table)

Study title: Patisiran (ALN-TTR02): An Embryo-Fetal Development Study by Intravenous Infusion in Rabbits

Study no.: TTR02-GLP15-034

Study report location: EDR

Conducting laboratory and location: (b) (4)

Date of study initiation: November 12, 2015

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: ALN-TTR02, Lot L0501305,

Methods

Doses: 0, 0.1, 0.3, or 0.6 mg/kg

Frequency of dosing:	See study design
Dose volume:	12 mL/kg
Route of administration:	IV infusion (1 h)
Formulation/Vehicle:	Saline
Species/Strain:	Female NZW rabbit
Number/Sex/Group:	20/group
Satellite groups:	TK (9/group)
Study design:	ALN-TTR02 was administered on GD 7, 13, and 19. Main and TK group animals were necropsied on GD 29 and 20, respectively.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality and Clinical Signs

Animals were monitored twice daily for mortality or signs of morbidity. 1 HDF was found dead on GD21. No COD was not determined; the sponsor did not rule out a drug relationship. There were no other drug-related mortalities. Drug-related clinical signs included decreased fecal output in 6/25 HDF.

Body Weight and Food Consumption

Body weights and food consumption were recorded daily. Mean maternal body weight gain was reduced up to 41 and 73% in MDF and HDF, respectively. Food consumption was reduced by up to 35% in HDF.

Toxicokinetics

Increases in plasma C_{max} and AUC for ALN-18328 and DLin-MC3-DMA were greater than dose proportional. Increases in C_{max} and AUC for PEG_{2000}-C-DMG were generally dose-proportional.

ALN-18328						
	Day of Gestation 7			Day of Gestation 19		
siRNA (mg/kg)[a]	0.1	0.3	0.6	0.1	0.3	0.6
$t_{1/2}$ (h)[b]	ND	8.45	8.45	ND	9.42	36.1
t_{max} (h)[b]	1.083	1.083	1.083	1.083	1.083	1.083
C_{max} (µg/mL)	0.641	3.12	8.23	0.339	1.61	3.60
t_{last} (h)[b]	3.0	20	25	4.0	7.0	14
AUC_{last} (µg.h/mL)[b]	0.776	10.1	24.1	ND	3.76	10.0
AUC_{24h} (µg.h/mL)[b]	0.776	10.1	24.1	ND	3.76	10.0
AUC_{inf} (µg.h/mL)[b]	ND	8.07	43.4	ND	6.19	15.7
CL (mL/h/kg)[b]	ND	37.2	13.8	ND	48.4	38.1
V_{ss} (mL/kg)	ND	253	120	ND	487	1170
Dlin-MC3-DMA						
	Day of Gestation 7			Day of Gestation 19		
siRNA (mg/kg)[a]	0.1	0.3	0.6	0.1	0.3	0.6
$t_{1/2}$ (h)[b]	8.07	6.27	6.21	9.42	7.16	8.93
t_{max} (h)[b]	1.083	1.083	1.083	1.083	1.083	1.083
C_{max} (µg/mL)	3.62	19.7	46.8	1.41	11.7	19.9
t_{last} (h)[b]	25	25	25	25	25	25
AUC_{last} (µg.h/mL)[b]	19.7	94.1	228	6.51	29.9	57.0
AUC_{24h} (µg.h/mL)[b]	19.7	94.1	228	6.51	29.9	57.0
AUC_{inf} (µg.h/mL)[b]	22.1	98.9	239	7.76	31.9	63.4
CL (mL/h/kg)[b]	30.2	20.9	16.7	107	67.7	76.4
V_{ss} (mL/kg)	292	145	133	1020	464	772
PEG_{2000}-C-DMG						
	Day of Gestation 7			Day of Gestation 19		
siRNA (mg/kg)[a]	0.1	0.3	0.6	0.1	0.3	0.6
$t_{1/2}$ (h)[b]	5.87	5.27	5.32	6.50	5.00	5.23
t_{max} (h)[b]	1.389	1.389	1.542	1.083	1.083	1.542
C_{max} (µg/mL)	1.10	3.46	8.20	0.486	2.45	3.64
t_{last} (h)[b]	25	25	25	25	25	25
AUC_{last} (µg.h/mL)[b]	7.88	23.6	52.5	2.58	10.9	18.9
AUC_{24h} (µg.h/mL)[b]	7.88	23.6	52.5	2.58	10.9	18.9
AUC_{inf} (µg.h/mL)[b]	8.32	24.6	54.7	2.75	11.2	21.9
CL (mL/h/kg)[b]	9.62	9.38	8.42	29.5	20.8	21.0
V_{ss} (mL/kg)	63.7	55.0	49.3	209	98.1	100

DG: Day of Gestation ; ND: not determined.
[a] siRNA doses of 0.1, 0.3 and 0.6 mg/kg correspond to DLin-MC3-DMA doses of 0.65, 1.94 and 3.89 mg/kg, respectively, which were used to calculate the TK parameters for DLin-MC3-DMA, and corresponded to PEG_{2000}-C-DMG doses of 0.08, 0.23 and 0.46 mg/kg, respectively, which were used to calculate the TK parameters for PEG_{2000}-C-DMG.
[b] Time from start of infusion (SOI) was used to calculate the parameter

(Sponsor's Table)

Hematology and Clinical Chemistry

Blood samples were collected prior to the initiation of dosing and at scheduled necropsy from fasted animals. There were no drug effects on coagulation or hematology.

Dosing Solution Analysis

All dosing solutions were within 15% of their respective target concentrations.

Necropsy

There were no drug-related gross findings.

Cesarean Section Data

There were no drug effects on corpora lutea, implantations, fetal body weights, % postimplantation loss, male/female ratio, resorptions, or dead fetuses.

Offspring

There were no drug-related external, visceral, or skeletal malformations.

9.3 Prenatal and Postnatal Development

Study title: Patisiran (ALN-TTR02) and AF-011-18534: An Intravenous Developmental and Perinatal/Postnatal Reproduction Toxicity Study in Rats, Including a Postnatal Behavioral Functional Evaluation

Study no.:	TTR02-GLP16-003
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	April 14, 2016
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALN-TTR02, Lot 1160150, 97.5% AF-011-18534, Lot 1160164, 97.7%

Methods

Doses:	0, 0.15, 0.5, or 1.5 mg/kg (ALN-TTR02); 1.5 mg/kg AF-011-18534
Frequency of dosing:	See study design
Dose volume:	12 mL/kg
Route of administration:	IV infusion (1 h)
Formulation/Vehicle:	Saline
Species/Strain:	Female SD rats
Number/Sex/Group:	24/group
Satellite groups:	TK (10/group)
Study design:	Pregnant rats were administered test article on GD 7, 13, and 19, and LD 6, 12, and 18.
Deviation from study protocol:	No significant deviations

Observations and Results

F_0 Dams

Survival: Animals were monitored twice daily for mortality or signs of morbidity. All animals survived until scheduled necropsy.

Clinical signs: There were no drug-related clinical signs.

Body weight: Body weights were recorded daily. There were no drug-related effects on body weight.

Food consumption: Food consumption was recorded on GD 7, 10, 12, 15, 18, 20, and 25, and on LD 1, 4, 7, 10, 12, and 14. There were no drug-related effects on food consumption.

Uterine content: There were no drug effects on litter parameters.

Necropsy observation: There were no drug-related gross findings.

Toxicokinetics: _Plasma_

Increases in C_{max} and AUC for ALN-18328, DLin-MC3-DMA, and PEG2000-C-DMG were less than dose proportional after dosing on LD 18.

LD 18									
	ALN-18328			DLin-MC3-DMA			PEG_{2000}-C-DMG		
siRNA (mg/kg)[a]	0.15	0.5	1.5[c]	0.15[c]	0.5[c]	1.5[c]	0.15[c]	0.5[c]	1.5[c]
$t_{1/2}$ (h)[b]	ND	ND	ND	66	ND	62	ND	37	11
t_{max} (h)[b]	2.00	1.08	1.08	2.00	1.08	3.00	1.08	1.08	2.00
C_{max} (ng/mL)	488	1200	3140	4020	2840	2820	621	1350	2820
AUC_{last} (ng·h/mL)[b]	1020	1500	6180	19100	27900	72500	3420	5360	15100
C_{max}/Dose[a]	3250	2400	2090	4360	922	306	5500	3600	2500
AUC_{last}/Dose[a,b]	6800	3000	4120	20700	9060	7850	30300	14300	13400

LD: lactation day; ND: not determined.

[a] siRNA (Patisiran) doses of 0.15, 0.5 and 1.5 mg/kg correspond to DLin-MC3-DMA doses of 0.923, 3.08 and 9.23 mg/kg, respectively, which were used to calculate the TK parameters for DLin-MC3-DMA, and correspond to PEG_{2000}-C-DMG doses of 0.113, 0.375 and 1.13 mg/kg, respectively, which were used to calculate the TK parameters for PEG_{2000}-C-DMG.

[b] Time-dependent parameters were calculated using times post start of infusion (SOI).

[c] TK parameter values were potentially impacted due to individual concentrations that were not quantifiable due to being above the limit of quantitation with insufficient sample volume for reassay, resulting in concentration -time profiles of $n < 3$ at the affected time points, most notably where $n = 0$ at 0.083 h post EOI. In most instances, the affected time points where concentrations were ALQ were at or around t_{max}.

(Sponsor's Table)

Milk

ALN-18328 and AD-18534 (siRNAs) were not detected in milk. Milk concentrations of DLin-MC3-DMA and PEG2000-C-DMG increased proportionally following administration of 0.5 or 1.5 mg/mL ALN-TTR02.

Patisiran Dose (mg/kg)	Mean[a] Concentration ± SD (ng/mL) on LD 12								
	ALN-18328			DLin-MC3-DMA			PEG_{2000}-C-DMG		
	Mean ± SD	n	NObs	Mean ± SD	n	NObs	Mean ± SD	n	NObs
0.15	NC	0	6	14.5 ± 2.91	5	6	NC	0	5
0.5	NC	0	6	50.0 ± 18.9	6	6	13.3 ± 0.265	3	6
1.5	NC	0	6	154 ± 80.1	6	6	45.8 ± 14.2	5	5

NC=not calculated – all individual animal concentration values < LLOQ; n=number of samples with detectible concentrations of ALN-18328, DLin-C-DMA or PEG_{2000}-C-DMG; NObs=number of observations (samples); SD=standard deviation.

Note: LLOQ was 400 ng/mL for ALN-18328, 10 ng/mL for DLin-MC3-DMA and PEG_{2000}-C-DMG.

[a] Means were calculated only from samples with quantifiable concentrations.

(Sponsor's Table)

Dosing Solution Analysis:	All dosing solutions were within 15% of their respective target concentrations.
Other:	There were no ALN-TTR02 mediated decreases in serum vitamin A, TTR, or T4. Administration of AF-011-18354 resulted in 82 and 66% decreases in serum vitamin A and T4, respectively, while TTR levels were reduced below the limit of quantification.

F_1 Generation

Survival:	Litters or weaned pups were observed at least twice daily for mortality or signs of morbidity.
Clinical signs:	There were no drug-related clinical signs.
Body weight:	Body weights were recorded on weekly during the postweaning period. There were no drug effects on body weight.
Food consumption:	Food consumption was recorded weekly until mating. There were no drug effects on food consumption.
Physical development:	There were no drug effects on physical development.
Neurological assessment:	Passive avoidance was evaluated by light/dark box on PND 24. Learning and memory were evaluated with a M-shaped water maze on PND 70. There were no drug effects on behavior.
Reproduction:	There were no drug effects on sexual maturation or estrous cycle. Animals within groups were paired for mating on PND 90. There were no drug effects on fertility index. Females were euthanized on GD 13; there were no drug effects on uterine or fetal parameters.
Other:	N/A

10 Special Toxicology Studies

Study title: *In Vitro* Evaluation of the Influence of ALN-TTR02 on Human Whole Blood Hemolysis and Plasma Flocculation

Study no.:	TTR-NCD10-017
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	January 6, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALN-TTR02, Lot IC118, 93.4%

Findings:
Concentrations of ALN-TTR02 up to 400 μg/mL did not cause hemolysis or turbidity in human whole blood.

Study title: Evaluation of Interferon-Alpha and Tumor Necrosis Factor-Alpha Secretion by Human Peripheral Blood Mononuclear Cells Following Transfection of Human TTR-Targeting siRNA (ALN-18328)

Study no.:	BIO09006
Study report location:	EDR
Conducting laboratory and location:	Alnylam Pharmaceuticals 300 Third Street Cambridge, MA 02142
Date of study initiation:	April 16, 2009
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	ALN-18328 (siRNA), Batch 1, 95.31%

Findings:
ALN-18328, the siRNA component of ALN-TTR02, did not induce IFN-α or TNF-α following transfection in isolated human PBMC.

Study title: Evaluation of the Secretion of Cytokines/Chemokines by Human Peripheral Blood Mononuclear Cells Following Transfection of ALN-18328, the Transthyretin-Targeting siRNA Component of ALN-TTR01

Study no.:	BIO09042
Study report location:	EDR
Conducting laboratory and location:	Alnylam Pharmaceuticals 300 Third Street Cambridge, MA 02142
Date of study initiation:	Not provided; study was conducted from August 11, 2009 to October 8, 2009.
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	ALN-18328 (siRNA), Batch 311086, 97.3%

Findings:

ALN-18328, the siRNA component of ALN-TTR02, did not induce IFN-α, IP-10, IFN-γ, TNF-α, IL-6, IL-1Ra, or G-CSF following transfection in isolated human PBMC.

Study title: An Exploratory Study of Immune Stimulation in CD-1 Male Mice After a Single IV Dose of Either ALN-TTR01 or ALN-TTR02

Study no.:	TTR02-NCD11-001
Study report location:	EDR
Conducting laboratory and location:	Alnylam Pharmaceuticals 300 Third Street Cambridge, MA 02142
Date of study initiation:	Not provided; study was conducted from February 17, 2011 to February 25, 2011.
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	ALN-TTR02, Lot IC118

Findings:

Male CD-1 mice (5/group/timepoint) were administered a single dose of 0, 7.5, or 15 mg/kg ALN-TTR01 or ALN-TTR02 by IV bolus; however, ALN-TTR01 was not discussed in the report. There was no drug-related mortality or morbidity. IL-6, IP-10, KC, MCP-1, and G-CSF were increased in a dose-dependent manner. Marked increases in mean ALT and AST occurring at 24 h postdose in HDM were due to excessive elevations in 2/5 animals.

Dose (mg/kg	Time Postdose (h)	ALT (U/L)	AST (U/L)	G-CSF (pg/mL)	IL-6 (pg/mL)	IP-10 (pg/mL)	KC (pg/mL)	MCP-1 (pg/mL)
0	2	20.97	65.73	655.15	19.53	180.51	87.12	78.13
	4	32.43	86.58	1339.67	19.53	146.23	122.49	78.13
	24	18.23	50.24	133.31	19.53	19.53	19.53	78.13
7.5	2	24.96	62.53	331.70	349.75	1348.69	1347.07	287.28
	4	33.84	85.40	4267.33	377.27	529.47	1594.34	645.54
	24	25.81	76.76	8493.71	215.07	699.65	111.78	307.18
15	2	35.10	83.37	745.18	1784.67	3494.73	3242.93	1574.85
	4	47.44	126.99	14835.41	1190.81	2376.89	2042.70	1564.30
	24	2602.46	3149.59	20000.00	815.41	2440.54	336.81	3946.66

11 Integrated Summary and Safety Evaluation

Introduction
ALN-TTR02 (patisrian-LNP) is a lipid nanoparticle (LNP)-encased siRNA developed by Alnylam Pharmaceuticals for the treatment of hereditary transthyretin-mediated amyloidosis. The LNP is thought to facilitate tissue-specific uptake of the drug product by the liver, after which the siRNA is intended to silence WT and mutant TTR mRNA. The proposed dosing for humans is 0.3 mg/kg IV every 3 weeks.

Pharmacology
Primary Pharmacology
The siRNA component of ALN-TTR02 is encapsulated in a LNP composed of two novel excipients, DLin-MC3-DMA and PEG_{2000}-C-DMG, as well cholesterol and the synthetic phosphatidylcholine 1,2-distearolyl-sn-Glycerol-3-phosphocholine (DSPC). According to the sponsor, upon IV administration of ALN-TTR02, PEG_{2000}-C-DMG dissociates from the drug product, allowing endogenous apolipoprotein E (ApoE) to bind with the LNP and facilitate low density lipoprotein receptor-mediated uptake by hepatocytes. Following hepatic uptake, the LNP becomes positively charged and disintegrates in the hepatocyte cytosol, releasing the siRNA that silences WT and mutant TTR mRNA. ALN-18328, the siRNA component of ALN-TTR02, is complimentary to a sequence of TTR mRNA conserved in monkeys and humans, but not rodents or rabbits. Studies with ALN-TTR01, which according to the sponsor is ALN-18328 formulated with an earlier and less-potent LNP composition, indicated an ED_{50} of 1 mg/kg for TTR mRNA silencing in cynomolgus monkeys, and reductions in serum TTR protein of up to 90 and 30% on postdose days 14 and 28, respectively, after administration of a single IV infusion of 0.3 mg/kg ALN-TTR01. Additionally, a study using a transgenic mouse model of TTR-amyloidosis (H129-hTTR V30M/Hsf-1 KO) indicated significant reductions in TTR protein immunoreactivity in esophagus, colon, stomach, sciatic nerve, and dorsal ganglion following six, twice-weekly IV bolus injections of 3 mg/kg ALN-TTR01. Using ALN-TTR02, single IV dosing up to 0.3 mg/kg in cynomolgus monkeys resulted in a 94% decrease in hepatic TTR mRNA and 80 and 70% decreases in serum TTR protein on Days 14 and 28, respectively. IV infusion of 0.15, 0.2, 0.25, or 0.3 mg/kg ALN-TTR02 monthly or every 3 weeks for 7 or 8 doses resulted in dose-dependent decreases of up to 95% in serum TTR in cynomolgus monkeys, with greater suppression occurring after each dose until the third or fourth dose.

Secondary Pharmacology
Seven potential off-target human transcripts were identified, possessing between 3 and 5 base mismatches compared to the target sequence on the TTR gene; however, *in vitro* assays in transfected HepB3 cells indicated that the affinity of ALN-18328 for each potential transcript was less than 1000-fold than that for TTR. Because renal excretion of retinol binding protein (RBP) is prevented by RBP/TTR binding, the effects of ALN-TTR02 on circulating RBP levels were also evaluated in cynomolgus monkeys administered a single 15 min IV infusion of 0 or 3 mg/kg. Circulating TTR and RBP were measured on Days 0 (baseline) and 7; the data indicated an approximately 50% reduction in both proteins. Additionally, vitamin A levels were assessed in male and female cynomolgus monkeys administered 0, 0.3, 1, or 3 mg/kg ALN-TTR02 by 1 h IV

infusion every 3 weeks for 39 weeks; the data indicated reductions of up to 81% of baseline levels during the dosing period. Because TTR transports thyroxine (T_4), serum T_4 levels were also assessed in the 39-week study; T_4 levels were reduced up to 50 and 37% in HDM and HDF, respectively, during the dosing period. However, vitamin A and T_4 levels returned to control levels over a 13-week recovery period. Based on the provided pharmacology studies, off target effects of ALN-TTR02 administration may include decreases in circulating RBP, vitamin A, and T_4 levels.

Safety Pharmacology
Safety pharmacology studies in transfected HEK cells and telemetered male cynomolgus monkeys evaluated the effects of ALN-TTR02 on hERG channel inhibition, and on CNS, cardiovascular, and respiratory function, respectively. The IC_{50} for hERG inhibition by ALN-TTR02 was higher than 1.5 mg/mL (highest dose tested). Telemetered male cynomolgus monkeys were administered ALN-TTR02 either as a single 1 h IV infusion of 0, 0.1, 1, 3, or 6 mg/kg to evaluate cardiac effects or a 1 h IV infusion of 3 mg/kg to evaluate CNS and respiratory effects. There were no drug effects on mean arterial pressure, QTc intervals, or ECG waveforms or rhythm; however, increases in HR between 30 to 120 BPM and 10 to 80 BPM were observed in 1/3 MHDM (from 4 to 48 h) and 1/3 HDM (from 4 to 34 h), respectively. There were no drug-related effects on behavior, motor function, cranial nerves, proprioception, respiratory rates, O_2 or CO_2 partial pressures, blood pH, or O_2 saturation. The safety pharmacology studies were adequately conducted and did not suggest a risk of QT prolongation or CNS or respiratory effects; however, dosing with ALN-TTR02 may result in transient increases in HR.

Excipients and Impurities
The LNP is composed of DLin-MC3-DMA, PEG_{2000}-C-DMG, DSPC, and cholesterol. All excipients were present in the ALN-TTR02 batches used in general, reproductive and developmental, and genetic toxicology studies. The sponsor was informed in EOP2 meeting minutes (October 22, 2013) that additional nonclinical studies would not be necessary to qualify the novel excipients DLin-MC3-DMA and PEG_{2000}-C-DMG. Additionally, DLin-MC3-DMA and PEG_{2000}-C-DMG were both negative when individually assessed in *in vitro* Ames and chromosomal aberration assays. Based on discussion with the CMC team, degradation products arising from DLin-MC3-DMA and PEG_{2000}-C-DMG are not of concern based on sufficiently similarity to their respective parent compounds. According to the sponsor, based on a review of the IID, the daily dose of DSPC is below that which would occur following chronic IV administration of other FDA-approved drugs and is, therefore, acceptable. Additionally, the proposed dosing regimen for patisiran would result in a daily dose of cholesterol that is approximately 0.6% of typical circulating endogenous levels.

Synthesis-related impurities in the ALN-TTR02 drug product included several genotoxic compounds; however, the sponsor will control such impurities to levels resulting in acceptable daily intakes based on chronic, intermittent dosing adjusted for a less-than lifetime exposure. Impurities related to the siRNA consisted of nucleotide additions or

deletions which are expected to have minimal pharmacological activity or be rapidly degraded. There are no nonclinical concerns regarding excipients or impurities.

PK/ADME
PK/ADME studies were primarily conducted in rat and monkey. Repeat IV dosing in both species did not result in plasma accumulation of ALN-18328, DLin-MC3-DMA, or PEG_{2000}-C-DMG. IV injection of radiolabeled ALN-TTR02 in rats resulted in maximal liver radioactivity within 4 h after dosing, with liver radioactivity accounting for 90% of the administered dose. Degradation of ALN-18328 was thought to be mediated by endonucleases in mice, rats, monkeys, and humans. Although PEG_{2000}-C-DMG was not metabolized in rat, monkey, or human, DLin-MC3-DMA was hydrolyzed through an unknown pathway in all three species to 4-(dimethylamino)butyric acid (DMBA). According to the sponsor, circulating DMBA was not present in rats or monkeys at levels greater than 10% of total DLin-MC3-DMA. Such data were not available for humans; however, the Clinical Pharmacology team indicated that DMBA is not likely to be a major circulating metabolite in humans. [redacted] (b) (4) the CMC team indicated minimal concern over this molecule based on its structural similarity to DLin-MC3-DMA. Therefore, no additional studies are needed to assess DMBA. In monkeys, PEG_{2000}-C-DMG and DLin-MC3-DMA (and DMBA) were excreted through the biliary and renal routes, respectively.

Toxicology
General toxicology studies were conducted in SD rats and cynomolgus monkeys. ALN-TTR02 was generally well tolerated by both species. Primary toxicities in male and female rats administered 0, 0.3, 0.1, or 0.3 mg/kg ALN-TTR02 once every 2 weeks for 26 weeks by 1 h IV infusion included 27 and 41% decreases in reticulocytes in MDM and HDM, respectively, 20 and 30% increases in fibrinogen in MDF and HDF, respectively, and increased occurrence of hepatocyte vacuolation in HDM and HDF. Drug-related findings resolved over a 12-week recovery period. Additional findings in rats included the formation of anti-PEG antibodies in 46 to 49% of animals at all doses and were thought to be responsible for levels of plasma ALN-18328 that were below detection limits on Day 183. The hepatocyte vacuolation was classified by the study pathologist as "minimal" and not associated with degenerative changes in the liver or elevations in liver function tests (LFT), and was, therefore, not considered by the sponsor to be adverse. The NOAEL in rats was 0.3 mg/kg (ALN-18328 C_{max} =2.4 μg/mL, AUC_{inf} = 4.4 $\mu g \times h/mL$; DLin-MC3-DMA C_{max} = 18 μg/mL, AUC_{inf} = 67.5 $\mu g \times h/mL$; PEG_{2000}-C-DMG C_{max} = 2.2 μg/mL, AUC_{inf} = 6.98 $\mu g \times h/mL$).

Primary toxicities in male and female cynomolgus monkeys administered 0, 0.3, 1, or 3/2 mg/kg ALN-TTR02 once every 2 weeks for 39 weeks by 1 h IV infusion consisted of elevations in LFT (up to 47-fold for ALT), especially in HDM, which resulted in a reduction of the HD in both sexes to 2 mg/kg. Histological observations correlating with elevations in LFT included centrilobular vacuolation (MDM, HDM, MDF, HDF), single cell necrosis (MDM, HDM, HDF), reactive sinusoid cells (HDM, HDF), mixed cell infiltration (MDM, HDM, HDF), and pigment deposition (MDM, HDM, MDF, HDF). Apart from pigment deposition, elevations in LFT and corresponding histopathology resolved

over a 13-week recovery period. There were no indications that ALN-TTR02 initiated complement or induced cytokine release. It was unclear why liver toxicity was more pronounced in males since exposure (C_{max} and AUC) to ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG was similar between sexes. The NOAEL in monkeys was 0.3 mg/kg based on liver toxicity (ALN-18328 C_{max} = 4.6 μg/mL, AUC_{inf} = 23.9 μg×h/mL; DLin-MC3-DMA C_{max} = 38.1 μg/mL, AUC_{inf} = 2085 μg×h/mL; PEG_{2000}-C-DMG C_{max} = 4.34 μg/mL, AUC_{inf} = 78.9 μg×h/mL).

Genetic Toxicology
ALN-TTR02, DLin-MC3-DMA, and PEG_{2000}-C-DMG were negative in an OECD-compliant battery of Ames and *in vitro* chromosomal aberration assays. ALN-TTR02 was also negative in an adequately-conducted *in vivo* mouse micronucleus assay.

Carcinogenicity
The sponsor was granted a waiver for a 2-year carcinogenicity study in rodents based on reductions in drug exposure secondary to the formation of ADA in the 26-week pivotal toxicology study. Carcinogenicity was, therefore, evaluated in a 26-week study in transgenic TgRasH2 mice, according to recommendations provided by the Executive CAC. Administration of 0 (saline), 0.5, 2, or 6 mg/kg ALN-TTR02 once every two weeks by IV injection was well tolerated. The 6-month study was adequately conducted, and an analysis conducted by the CDER Office of Biometrics did not indicate a significant effect of ALN-TTR02 on survival or tumor formation.

Reproductive Toxicology
Reproductive toxicity was adequately assessed in fertility, embryofetal development, and pre- and postnatal development studies in SD rats and in an embryofetal development study in NZW rabbits. There were no drug effects on mating or sperm parameters in male SD rats administered up to 0.3 mg/kg ALN-TTR02 or 0.1 mg/kg of the rodent surrogate AF-011-18534 by IV infusion every other week for 10 weeks prior to cohabitation, and continuing through the mating period for a total of 7 doses. There were no drug-related fetal malformations or effects on pregnancy or uterine parameters following administration of up to 1.5 mg/kg ALN-TTR02 or AF-011-18534 by weekly IV infusion beginning two weeks prior to mating and continuing until GD 19 in female SD rats. In a dose-ranging study in NZW rabbits, administration of 1 or 2 mg/kg ALN-TTR02 resulted in loss of litters that was thought to be secondary to maternal toxicity (i.e., weight loss and liquid feces). In the pivotal embryofetal development study in NZW rabbits, administration of ALN-TTR02 (0, 0.1, 0.3, or 0.6 mg/kg) on GD 7, 13, and 19 resulted in weight loss in MDF and HDF. There was one HDF fatality on GD 21; a COD could not be determined. Necropsy of the remaining animals on GD 29 did not indicate drug-related fetal malformations or effects on pregnancy or uterine parameters. In a pre- and postnatal development study, there were no drug effects on physical, neurobehavioral, or reproductive development of offspring following weekly 1 h IV infusion of 0, 0.15, 0.5, or 1.5 mg/kg ALN-TTR02 or 1.5 mg/kg AF-011-18534 to pregnant SD rats from GD 7 until LD 18. Collectively, there were no reproductive or developmental toxicities associated with ALN-TTR02 in rats or rabbits.

Hemolysis and Immunogenicity

Incubation of up to 400 μg/mL ALN-TTR02 with human whole blood did not cause hemolysis or turbidity. Transfection of isolated human peripheral blood mononuclear cells with ALN-18328 and administration of up to 15 mg/kg ALN-TTR02 by single IV bolus in male CD-1 mice did not result in secretion of inflammatory cytokines.

Conclusions

The nonclinical studies supporting this NDA were adequately conducted. Chronic toxicity studies indicated a risk for liver injury based on findings in rats and monkeys. However, such findings are reversible and can be clinically monitored. The nonclinical data support approval of ALN-TTR02.

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/s/

DAVID L CARBONE
07/11/2018

LOIS M FREED
07/11/2018

Center for Drug Evaluation and Research Division of Cardiovascular and Renal Products

DCRP Consult Addendum NDA 210922

ADDENDUM DATE: 7/3/2018

FROM: Preston M. Dunnmon, M.D., M.B.A., Medical Officer
Division of Cardiovascular and Renal Products, HFD-110

THROUGH: Martin Rose, M.D., J.D., Medical Team Leader
Division of Cardiovascular and Renal Products, HFD-110

Norman Stockbridge, M.D., Ph.D., Division Director
Division of Cardiovascular and Renal Products, HFD-110

TO: Nick Kozauer, MD, CDTL
Division of Neurology Products, HFD-120

DRUG NAME: Onpattro (Patisiran-LPN, ALN-TTR02)

DOSE/FORMULATION: Lipid nanoparticle formulation (Patisiran-LNP) for IV administration every 3 weeks at 0.3 mg/kg over approximately 80 minutes, to be preceded by premedication with a corticosteroid, acetaminophen and antihistamines

MECH OF ACTION: A double-stranded small interfering ribonucleic acid (siRNA) targeting a conserved region in the 3' untranslated region (UTR) of wt and mutant TTR mRNA

APPLICANT: Alnylam Pharmaceuticals

CONSULT QUESTION: The DNP requests that DCRP review post-hoc CV outcomes analyses submitted [redacted] (b) (4)

DOCUMENT REVIEWED:

- Post-hoc overview of CV outcomes from trial ALN-TTR02-004 (APOLLO Phase 3, N=148 patisiran-LPN, N=77 placebo, 18 months)

(b) (4) [redacted]

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Assessment

These are interesting trends from a post-hoc analysis of a single small study with a small number of outcome events in a population of polyneuropathy-predominant patients (b) (4) [redacted]

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/s/

PRESTON M DUNNMON
07/05/2018

MARTIN ROSE
07/06/2018

NORMAN L STOCKBRIDGE
07/06/2018

Medical Officer's Review of NDA 210922
Ophthalmology Consultant

NDA 210-922	Submission Date:	12/11/2017
Consult Review	Consult Request Date:	2/22/2018
	Review completed:	6/18/2018

Product Name: Onpattro (patisiran) injection 2mg/mL

Class: Small interfering RNA (siRNA) molecule that reduces the levels of the TTR protein

Proposed indication: Treatment of adults with hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis)

Sponsor: Alnylam Pharmaceuticals

Requested from the Division of Neurology Products: This application is for patisiran for the treatment of hereditary transthyretin amyloidosis (hATTR) [redacted] (b) (4). This drug is a small interfering RNA (siRNA) molecule that reduces the levels of the TTR protein, which also transports retinol (vitamin A) to tissues. The main evidence of efficacy comes from Study 004 (APOLLO). Subjects received vitamin A supplementation in this development program. The applicant's discussion of ocular safety can be found on pages 95-96 of the ISS. Ocular abnormalities are also frequently observed due to the underlying disease.

1. Please evaluate the clinical study adverse event data and electroretinogram (ERG) data for evidence of ocular toxicity related to vitamin A deficiency.
2. Please comment on whether the applicant adequately evaluated for ocular toxicity related to vitamin A deficiency in the clinical studies of patisiran. If applicable, provide recommendations for further evaluation.
3. Please comment on the adequacy of the applicant's proposed labeling to reduce the potential risk of ocular toxicity due to vitamin A deficiency.

Ophthalmology Preliminary Review: A preliminary review of the ocular data was performed. The following issues and requests for information was forwarded to the applicant.

Study ALN-TTR02-003

1. Ophthalmology examinations were described in Section 7.2.4 of the protocol. Details regarding the ophthalmology examinations were to be provided in the Study Reference Manual. The Study Reference Manual has not been located in the NDA submission. Please submit the Study Reference Manual or identify its specific location in the NDA submission.

2. Manual Biomicroscopy (slit lamp) and dilated indirect ophthalmoscopy examinations were classified as normal, abnormal/not clinically significant and abnormal/clinically significant). Please describe any instructions given to the investigator for classifying these events.

3. There are individual patient details reported for Patient [redacted] (b) (6). These details include: "On Day 749, the patient reported decreased visual acuity at night (Appendix 16.2.7.1), and on Day 752 papilloedema was reported on exam. The decreased visual acuity was considered unlikely related to study drug and the papilloedema was considered possibly related to study drug (Appendix 16.2.7.1). The patient had no symptoms of increased intracranial pressure such as headaches, nausea or vomiting (Appendix 16.2.7.1). The patient was referred to an ophthalmologist. Electroretinography testing was normal. Visual field testing was stable (Appendix 16.2.8.14). The relevant portion of the table has been reproduced below:

[redacted] (b) (6)	SCREENING/BASELINE	[redacted] (b) (6)	-4	MEAN DEVIATION	LEFT	-2.83
					RIGHT	-2.94
	WEEK 27		186	MEAN DEVIATION	LEFT	-3.41
					RIGHT	-2.33
	WEEK 54		374	MEAN DEVIATION	LEFT	-2.81
					RIGHT	-1.53
	WEEK 81		591	MEAN DEVIATION	LEFT	-4.98
					RIGHT	-6.35
	WEEK 108		752	MEAN DEVIATION	LEFT	-5.99
					RIGHT	-7.36

The patient was noted to have worsening of his cataracts, which was considered normal for his age and most likely responsible for his difficulty with visual acuity at night per the ophthalmologist.

Considering that the reason for ophthalmologic monitoring was a possible association between use of the drug product and retinal function, please explain why decreased visual acuity at night would be considered unlikely to be related and why the visual field testing was considered stable when it was decreasing.

4. Treatment-emergent abnormal fundus findings that were considered clinically significant were recorded in the adverse event log. The following ocular events are listed:

Eye disorders/Corneal opacity/ DISCRETE HAZE IN RIGHT EYE CORNEA	[redacted] (b) (6)	3	[redacted] (b) (6)	14:00/	747/	DOSE NOT CHANGED	NOT RECOVERED/ NOT RESOLVED	MILD	UNLIKELY RELATED
Eye disorders/ Macular degeneration/ MACULAR DEGENERATION LEFT EYE		17			529/ 532	DOSE NOT CHANGED	RECOVERED/ RESOLVED WITH SEQUELAE	MODERATE	NOT RELATED
Eye disorders/ Macular degeneration/ MACULAR DRUSEN BILATERAL		27			376/	DOSE NOT CHANGED	NOT RECOVERED/ NOT RESOLVED	MILD	NOT RELATED
Eye disorders/Macular degeneration/ RIGHT MACULAR DRUSEN		6			374/ 563	DOSE NOT CHANGED	RECOVERED/ RESOLVED	MILD	NOT RELATED
Eye disorders/Macular fibrosis/ EPIRETINALT MEMBRANE LEFT EYE		18			478/ 532	DOSE NOT CHANGED	RECOVERED/ RESOLVED	MILD	NOT RELATED

Eye disorders/Macular fibrosis/ RIGHT EYE EPIRETINEAL MEMBRAN	[redacted] (b) (6)	5	[redacted] (b) (6)		592/	DOSE NOT CHANGED	NOT RECOVERED/ NOT RESOLVED	MODERATE	NOT RELATED
Eye disorders/Vision blurred/ SLIGHTLY BLURRED VISION		21			392/	DOSE NOT CHANGED	NOT RECOVERED/ NOT RESOLVED	MILD	UNLIKELY RELATED
Eye disorders/Visual acuity reduced/ PATIENT EXPERIENCE LOW VISION LEFT EYE		22		8:00/	379/ 532	DOSE NOT CHANGED	RECOVERED/ RESOLVED	MODERATE	UNLIKELY RELATED
Eye disorders/ Visual acuity reduced/ DECREASE NIGHT VISUAL ACUITY		37			749/	DOSE NOT CHANGED	NOT RECOVERED/ NOT RESOLVED	MODERATE	UNLIKELY RELATED

Considering that the reason for ophthalmologic monitoring was a possible association between use of the drug product and retinal function and/or corneal function, please explain why the events listed above would be considered not related or unlikely to be related. In addition, please explain how the epiretinal membrane resolved. Please explain why the additional events in the database and considered abnormal and clinically significant for subjects [redacted] (b) (6) and [redacted] (b) (6) were not included in this listing.

[redacted] (b) (6)	MACULA	DILATED INDIRECT OPHTHALMOSCOPY	ABNORMAL, CLINICALLY SIGNIFICANT	EYE, RIGHT	WEEK 54	[redacted] (b) (6)
	RETINA	DILATED INDIRECT OPHTHALMOSCOPY	ABNORMAL, CLINICALLY SIGNIFICANT	EYE, RIGHT	WEEK 54	
	LENS	SLIT LAMP BIOMICROSCOPY	ABNORMAL, CLINICALLY SIGNIFICANT	EYE, LEFT	WEEK 81	
	LENS	SLIT LAMP BIOMICROSCOPY	ABNORMAL, CLINICALLY SIGNIFICANT	EYE, RIGHT	WEEK 81	
	RETINA	DILATED INDIRECT OPHTHALMOSCOPY	ABNORMAL, CLINICALLY SIGNIFICANT	EYE, RIGHT	WEEK 81	
	LENS	SLIT LAMP BIOMICROSCOPY	ABNORMAL, CLINICALLY SIGNIFICANT	EYE, LEFT	WEEK 108	
	LENS	SLIT LAMP BIOMICROSCOPY	ABNORMAL, CLINICALLY SIGNIFICANT	EYE, RIGHT	WEEK 108	
	RETINA	DILATED INDIRECT OPHTHALMOSCOPY	ABNORMAL, CLINICALLY SIGNIFICANT	EYE, LEFT	WEEK 108	
	RETINA	DILATED INDIRECT OPHTHALMOSCOPY	ABNORMAL, CLINICALLY SIGNIFICANT	EYE, RIGHT	WEEK 108	
	MACULA	DILATED INDIRECT OPHTHALMOSCOPY	ABNORMAL, CLINICALLY SIGNIFICANT	EYE, RIGHT	WEEK 54	
	MACULA	DILATED INDIRECT OPHTHALMOSCOPY	ABNORMAL, CLINICALLY SIGNIFICANT	EYE, RIGHT	WEEK 81	
	MACULA	DILATED INDIRECT OPHTHALMOSCOPY	ABNORMAL, CLINICALLY SIGNIFICANT	EYE, RIGHT	WEEK 108	

5. The submitted database for the ocular examination appears to have a number of errors. Please explain or correct the following:

   a. Seeing a single letter on a LOGMAR scale is 0.02. Scores that are not a multiple of 0.02 are suggestive of errors.

ALN-TTR02-003	(b) (6)	LMS	LOGMAR SCORE	VISUAL ACUITY	-0.09		EYE, RIGHT	WEEK 108	(b) (6)
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	-0.09		EYE, RIGHT	WEEK 108	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	-0.09		EYE, LEFT	WEEK 27	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	-0.09		EYE, LEFT	WEEK 108	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	-0.09		EYE, RIGHT	WEEK 27	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	-0.09		EYE, LEFT	WEEK 27	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	-0.09		EYE, RIGHT	WEEK 27	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	0.001		EYE, LEFT	WEEK 54	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	0.001		EYE, RIGHT	WEEK 54	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	0.09		EYE, LEFT	WEEK 108	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	0.09		EYE, LEFT	WEEK 81	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	0.09		EYE, LEFT	WEEK 81	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	0.09		EYE, LEFT	SCREENING	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	0.09		EYE, LEFT	WEEK 27	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	0.15		EYE, LEFT	WEEK 54	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	0.71		EYE, RIGHT	WEEK 108	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	0.77		EYE, RIGHT	SCREENING	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	0.87		EYE, RIGHT	WEEK 81	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	0.93		EYE, LEFT	SCREENING	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	0.93		EYE, LEFT	WEEK 27	
ALN-TTR02-003		LMS	LOGMAR SCORE	VISUAL ACUITY	0.000.1		EYE, LEFT	SCREENING	

ALN-TTR02-003	[redacted] (b) (6)	LMS	LOGMAR SCORE	VISUAL ACUITY	0.000.1		EYE, LEFT	SCREENING	[redacted] (b) (6)
ALN-TTR02-003	[redacted]	LMS	LOGMAR SCORE	VISUAL ACUITY	--0.3		EYE, LEFT	WEEK 81	[redacted]
ALN-TTR02-003	[redacted]	LMS	LOGMAR SCORE	VISUAL ACUITY	--0.3		EYE, RIGHT	WEEK 81	[redacted]
ALN-TTR02-003	[redacted]	LMS	LOGMAR SCORE	VISUAL ACUITY	x6		EYE, LEFT	WEEK 108	[redacted]
ALN-TTR02-003	[redacted]	LMS	LOGMAR SCORE	VISUAL ACUITY	x6.5		EYE, RIGHT	WEEK 108	[redacted]

Cup to Disc ratio are normally estimated fractions to one tenth; values to one hundredth are suggestive of machine read values or potential errors. Values greater than 1 are impossible.

ALN-TTR02	[redacted] (b) (6)	CTD	CUP-TO-DISC RATIO	0.33	EYE, LEFT	WEEK 54	[redacted] (b) (6)
ALN-TTR02	[redacted]	CTD	CUP-TO-DISC RATIO	0.18	EYE, LEFT	WEEK 81	[redacted]
ALN-TTR02	[redacted]	CTD	CUP-TO-DISC RATIO	0.32	EYE, LEFT	WEEK 108	[redacted]
ALN-TTR02	[redacted]	CTD	CUP-TO-DISC RATIO	0.31	EYE, RIGHT	WEEK 54	[redacted]
ALN-TTR02	[redacted]	CTD	CUP-TO-DISC RATIO	0.15	EYE, RIGHT	WEEK 81	[redacted]
ALN-TTR02	[redacted]	CTD	CUP-TO-DISC RATIO	0.28	EYE, RIGHT	WEEK 108	[redacted]
ALN-TTR02	[redacted]	CTD	CUP-TO-DISC RATIO	0.21	EYE, LEFT	WEEK 54	[redacted]
ALN-TTR02	[redacted]	CTD	CUP-TO-DISC RATIO	0.31	EYE, LEFT	WEEK 108	[redacted]
ALN-TTR02	[redacted]	CTD	CUP-TO-DISC RATIO	0.19	EYE, RIGHT	WEEK 54	[redacted]
ALN-TTR02	[redacted]	CTD	CUP-TO-DISC RATIO	0.09	EYE, RIGHT	WEEK 81	[redacted]
ALN-TTR02	[redacted]	CTD	CUP-TO-DISC RATIO	0.28	EYE, RIGHT	WEEK 108	[redacted]
ALN-TTR02	[redacted]	CTD	CUP-TO-DISC RATIO	7	EYE, LEFT	WEEK 27	[redacted]
ALN-TTR02	[redacted]	CTD	CUP-TO-DISC RATIO	7	EYE, RIGHT	WEEK 27	[redacted]

Sphere and Cylinder Values are in quarters of a diopter. Plano is by definition a zero value. The meaning of PLANO 1 is not clear. The values listed below are likely errors.

ALN-TTR02	[redacted] (b) (6)	MRC	MANIFEST REFRACTION CYLINDER	0.78	EYE, RIGHT	WEEK 108	[redacted] (b) (6)
ALN-TTR02	[redacted]	MRC	MANIFEST REFRACTION CYLINDER	--0.5	EYE, LEFT	WEEK 81	[redacted]
ALN-TTR02	[redacted]	MRC	MANIFEST REFRACTION CYLINDER	--0.75	EYE, LEFT	WEEK 54	[redacted]
ALN-TTR02	[redacted]	MRC	MANIFEST REFRACTION CYLINDER	--0.75	EYE, RIGHT	WEEK 54	[redacted]
ALN-TTR02	[redacted]	MRS	MANIFEST REFRACTION SPHERE	-1.8	EYE, LEFT	WEEK 27	[redacted]
ALN-TTR02	[redacted]	MRS	MANIFEST REFRACTION SPHERE	-1.8	EYE, RIGHT	WEEK 27	[redacted]
ALN-TTR02	[redacted]	MRS	MANIFEST REFRACTION SPHERE	+PLANO 1	EYE, LEFT	WEEK 54	[redacted]
ALN-TTR02	[redacted]	MRS	MANIFEST REFRACTION SPHERE	+PLANO 1	EYE, RIGHT	WEEK 54	[redacted]
ALN-TTR02	[redacted]	MRS	MANIFEST REFRACTION SPHERE	PLANO 0.5	EYE, LEFT	WEEK 54	[redacted]
ALN-TTR02	[redacted]	MRS	MANIFEST REFRACTION SPHERE	-PLANO 1	EYE, LEFT	WEEK 54	[redacted]
ALN-TTR02	[redacted]	MRS	MANIFEST REFRACTION SPHERE	-PLANO 1	EYE, RIGHT	WEEK 54	[redacted]

6. Visual fields are not appropriately represented by mean deviation. Please submit copies of all visual fields.

Study ALN-TTR02-004

1. Ophthalmology examinations were described in Section 7.5.6 of the protocol. Details regarding the ophthalmology examinations were to be provided in the Study Reference Manual. The Study Reference Manual has not been located in the NDA submission. Please submit the Study Reference Manual or identify its specific location in the NDA submission.

2. Manual Biomicroscopy (slit lamp) and dilated indirect ophthalmoscopy examinations were classified as normal, abnormal/not clinically significant and abnormal/clinically significant). Please describe any instructions given to the investigator for classifying these events.

3. Electroretinograms were classified as normal, attenuated or extinguished. Please describe any instructions given to the investigator for classifying these events. Please submit all electroretinograms that were abnormal in any parameter.

4. Considering that the reason for ophthalmologic monitoring was a possible association between use of the drug product and retinal function and/or corneal surface conditions, please explain why the events listed above would be considered not related or unlikely to be related.

System Organ Class/Preferred Term/Verbatim	Patient Number	Age	Date of Event	Study Day	Outcome	Severity	Causal Relationship	Serious Criteria	Study Withdrawal
Eye disorders/Corneal Erosion/ EPITHELIAL EROSION OF INFERIOR CORNEA	(b) (6) [redacted]	4	(b) (6) [redacted]	219/557	Recovered Resolved	Mild	Not Related	N	N
Eye disorders/Corneal opacity/CORNEAL OPACITY		7		547	Not Recovered Not Resolved	Mild	Not Related	N	N
Eye disorders/Dry Eye/BILATERAL DRY EYES		8		200	Not Recovered Not Resolved	Mild	Not Related	N	N
Eye disorders/Dry Eye/DRY EYES		0		456	Not Recovered Not Resolved	Mild	Not Related	N	N
Eye disorders/Dry Eye/DRY EYES		6		184	Not Recovered Not Resolved	Mild	Unlikely Related	N	N
Eye disorders/Dry Eye/DRY EYES		1		499	Not Recovered Not Resolved	Mild	Not Related	N	N
Eye disorders/Dry Eye/DRY EYES		2		558	Not Recovered Not Resolved	Mild	Unlikely Related	N	N
Eye disorders/Dry Eye/DRY EYE		1		549	Not Recovered Not Resolved	Mild	Not Related	N	N
Eye disorders/Glaucoma/GLAUCOMA Retinal detachment/RETINAL DETACHMENT LEFT EYE		4		353/560 58/74	Not Recovered Not Resolved Recovered Resolved with Sequelae	Mild Moderate	Unlikely Related Not Related	N	N
Eye disorders/Glaucoma/GLAUCOMA WORSENING		2		557	Not Recovered Not Resolved	MILD	Unlikely Related	N	N
Eye disorders/Keratitis/KERATITIS		9		265/274	Recovered Resolved	MILD	Not Related	N	N
Eye disorders/Keratitis/KERATITE OXFORD 3		1		268	Not Recovered Not Resolved	MILD	Not Related	N	N
Eye disorders/Maculopathy/ PARACENTRAL ACUTE MIDDLE MACULOPATHY		0		462	Not Recovered Not Resolved	SEVERE	Unlikely Related	Y	N
Eye disorders/Ocular discomfort/DISCOMFORT OF BOTH EYES		1		171	Not Recovered Not Resolved	MILD	Not Related	N	N
Eye disorders/Optic atrophy/RIGHT EYE OPTICAL NERVE PALOR Retinal pigment epitheliopathy/POSSIBLE RPE CHANGES INTO THE MIDPERIPHERY BOTH EYES		2		254 554	Not Recovered Not Resolved	MILD	Not Related	N	N
Eye disorders/Optic nerve cupping/BILATERAL EXCAVATION OF		9		262	Not Recovered Not Resolved	MILD	Unlikely Related	N	N

OPTIC NERVE									
Eye disorders/Retinal Scar/LEFT RETINAL SCAR	(b) (6) [redacted]	68	(b) (6) [redacted]	275	Not Recovered Not Resolved	MILD	Not Related	N	N
Eye disorders/Vision Blurred/BLURRED VISION	[redacted]	61	[redacted]	334	Not Recovered Not Resolved	MILD	Not Related	N	N
Eye disorders/Vision Blurred/BLURRED VISION	[redacted]	66	[redacted]	159/167	Not Recovered Not Resolved	MILD	Not Related	N	N
Eye disorders/Vision Blurred/WORSENING VISION RIGHT EYE (BLURRED VISION)	[redacted]	74	[redacted]	340	Not Recovered Not Resolved	MILD	Not Related	N	N
Eye disorders/Vision Blurred/SLIGHT BLURRED VISION	[redacted]	65	[redacted]	160	Not Recovered Not Resolved	MILD	Not Related	N	N
Eye disorders/Visual impairment/VISUAL DISTURBANCE	[redacted]	65	[redacted]	43/262	Recovered Resolved	MILD	Not Related	N	N

5. The submitted database for the ocular examination appears to have a number of errors. Please explain or correct the following:
   a. Why are there no values less than 0?
   b. Seeing a single letter on a LOGMAR scale is 0.02. Scores that are not a multiple of 0.02 are suggestive of errors.

ALN-TTR02-004	(b) (6) [redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.09	3	BASELINE	(b) (6) [redacted]	-21
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.09	3	BASELINE	[redacted]	-7
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.09	3	BASELINE	[redacted]	-13
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.09	3	BASELINE	[redacted]	-13
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.09	3	BASELINE	[redacted]	-11
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.09	3	BASELINE	[redacted]	-18
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.09	3	BASELINE	[redacted]	-13
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.09	32	MONTH 18	[redacted]	562
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.09	32	MONTH 18	[redacted]	562
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.09	3	BASELINE	[redacted]	-15
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.09	32	MONTH 18	[redacted]	553
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.01	3	BASELINE	[redacted]	-15
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.09	32	MONTH 18	[redacted]	556
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.17	32	MONTH 18	[redacted]	555
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.17	32	MONTH 18	[redacted]	555
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.29	17	MONTH 9	[redacted]	252
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.15	3	BASELINE	[redacted]	-15
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.15	32	MONTH 18	[redacted]	559
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.15	17	MONTH 9	[redacted]	274
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.15	32	MONTH 18	[redacted]	559
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.15	17	MONTH 9	[redacted]	257
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.15	17	MONTH 9	[redacted]	270
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.15	17	MONTH 9	[redacted]	267
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.15	17	MONTH 9	[redacted]	267
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.15	3	BASELINE	[redacted]	-28
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.05	17	MONTH 9	[redacted]	263
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.09	3	BASELINE	[redacted]	-15
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.09	3	BASELINE	[redacted]	-15
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.09	3	BASELINE	[redacted]	-14
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.15	3	BASELINE	[redacted]	-11
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.99	17	MONTH 9	[redacted]	253
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.63	3	BASELINE	[redacted]	-63
ALN-TTR02-004	[redacted]	LogMar Score Left Eye	VISUAL ACUITY	0.55	3	BASELINE	[redacted]	-7
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.55	3	BASELINE	[redacted]	-7
ALN-TTR02-004	[redacted]	LogMar Score Right Eye	VISUAL ACUITY	0.63	17	MONTH 9	[redacted]	252

c. Cup to Disc ratio are normally estimated fractions to one tenth; values to one hundredth are suggestive of machine read values or potential errors. Values greater than 1 are impossible.

ALN-TTR02-004-	(b) (6) [redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.21	LEFT EYE	BASELINE	(b) (6) [redacted]	-19
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.14	LEFT EYE	MONTH 9	[redacted]	242
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.08	RIGHT EYE	MONTH 9	[redacted]	242
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.24	RIGHT EYE	MONTH 18	[redacted]	557
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.14	LEFT EYE	MONTH 9	[redacted]	276
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.18	LEFT EYE	MONTH 18	[redacted]	556
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.14	RIGHT EYE	MONTH 9	[redacted]	276
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.17	RIGHT EYE	MONTH 18	[redacted]	556
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.27	LEFT EYE	BASELINE	[redacted]	1
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.07	LEFT EYE	MONTH 9	[redacted]	267
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.44	LEFT EYE	MONTH 18	[redacted]	555
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.38	RIGHT EYE	BASELINE	[redacted]	1
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.11	RIGHT EYE	MONTH 9	[redacted]	267
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.58	RIGHT EYE	MONTH 18	[redacted]	555
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.28	LEFT EYE	BASELINE	[redacted]	-12
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.53	LEFT EYE	MONTH 9	[redacted]	248
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.42	LEFT EYE	MONTH 18	[redacted]	556
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.36	RIGHT EYE	BASELINE	[redacted]	-12
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.54	RIGHT EYE	MONTH 9	[redacted]	248
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.52	RIGHT EYE	MONTH 18	[redacted]	556
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	2.5	LEFT EYE	BASELINE	[redacted]	-21
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	2.5	LEFT EYE	MONTH 9	[redacted]	259
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	2.5	LEFT EYE	MONTH 18	[redacted]	552
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	3	RIGHT EYE	BASELINE	[redacted]	-21
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	3	RIGHT EYE	MONTH 9	[redacted]	259
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	3	RIGHT EYE	MONTH 18	[redacted]	552
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	6	LEFT EYE	BASELINE	[redacted]	-15
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	6	RIGHT EYE	BASELINE	[redacted]	-15
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	4	LEFT EYE	BASELINE	[redacted]	-14
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	3	RIGHT EYE	BASELINE	[redacted]	-14
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.05	LEFT EYE	MONTH 9	[redacted]	253
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.05	RIGHT EYE	MONTH 9	[redacted]	253
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	6.3	LEFT EYE	MONTH 18	[redacted]	557
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	6.3	RIGHT EYE	MONTH 18	[redacted]	557
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	6.35	LEFT EYE	MONTH 18	[redacted]	554
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	6.7	LEFT EYE	MONTH 9	[redacted]	273
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	6.5	LEFT EYE	MONTH 18	[redacted]	558
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	6.5	RIGHT EYE	MONTH 9	[redacted]	273
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	6.5	RIGHT EYE	MONTH 18	[redacted]	558
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.31	LEFT EYE	MONTH 9	[redacted]	253
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.05	LEFT EYE	BASELINE	[redacted]	-8
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.29	LEFT EYE	BASELINE	[redacted]	-1
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.29	LEFT EYE	MONTH 9	[redacted]	248
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.37	LEFT EYE	MONTH 18	[redacted]	556
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.31	RIGHT EYE	MONTH 18	[redacted]	556
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.44	LEFT EYE	BASELINE	[redacted]	-4
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.49	LEFT EYE	MONTH 18	[redacted]	550
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.53	RIGHT EYE	MONTH 9	[redacted]	249
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.46	RIGHT EYE	MONTH 18	[redacted]	550
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.58	LEFT EYE	MONTH 9	[redacted]	247
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.57	LEFT EYE	MONTH 18	[redacted]	548
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.62	RIGHT EYE	BASELINE	[redacted]	-20
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.56	RIGHT EYE	MONTH 9	[redacted]	247
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.48	LEFT EYE	MONTH 18	[redacted]	555
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.47	RIGHT EYE	BASELINE	[redacted]	2
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.47	RIGHT EYE	MONTH 18	[redacted]	555
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.67	LEFT EYE	BASELINE	[redacted]	2
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.67	LEFT EYE	MONTH 9	[redacted]	233

ALN-TTR02-004-	[redacted] (b) (6)	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.48	RIGHT EYE	MONTH 9	[redacted] (b) (6)	233
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.43	LEFT EYE	MONTH 9	[redacted]	232
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.46	LEFT EYE	MONTH 18	[redacted]	547
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.44	RIGHT EYE	MONTH 9	[redacted]	232
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.46	RIGHT EYE	MONTH 18	[redacted]	547
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.41	LEFT EYE	BASELINE	[redacted]	-14
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.41	LEFT EYE	MONTH 9	[redacted]	253
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.42	LEFT EYE	MONTH 18	[redacted]	554
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.41	RIGHT EYE	BASELINE	[redacted]	-14
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.41	RIGHT EYE	MONTH 9	[redacted]	253
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.61	LEFT EYE	MONTH 18	[redacted]	549
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.46	LEFT EYE	BASELINE	[redacted]	3
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.51	RIGHT EYE	BASELINE	[redacted]	3
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.36	LEFT EYE	BASELINE	[redacted]	-8
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio RE	DILATED INDIRECT OPHTHALMOSCOPY	0.44	RIGHT EYE	BASELINE	[redacted]	-8
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.33	LEFT EYE	BASELINE	[redacted]	-8
ALN-TTR02-004-	[redacted]	Cup-To-Disc Ratio LE	DILATED INDIRECT OPHTHALMOSCOPY	0.33	LEFT EYE	MONTH 9	[redacted]	254

d. Sphere and Cylinder Values are in quarters of a diopter. The values listed below are likely errors. In addition, there were no values less than zero. This is likely an error.

ALN-TTR02-004	[redacted] (b) (6)	Manifest Refraction Cylinder Left Eye	0.9	LEFT EYE	MONTH 18	[redacted] (b) (6)	561
ALN-TTR02-004	[redacted]	Manifest Refraction Cylinder Left Eye	1.8	LEFT EYE	MONTH 9	[redacted]	267
ALN-TTR02-004	[redacted]	Manifest Refraction Sphere Right Eye	0.1	RIGHT EYE	MONTH 18	[redacted]	565
ALN-TTR02-004	[redacted]	Manifest Refraction Sphere Right Eye	0.15	RIGHT EYE	MONTH 18	[redacted]	548
ALN-TTR02-004	[redacted]	Manifest Refraction Sphere Left Eye	0.2	LEFT EYE	BASELINE	[redacted]	-20
ALN-TTR02-004	[redacted]	Manifest Refraction Sphere Right Eye	0.2	RIGHT EYE	BASELINE	[redacted]	-20
ALN-TTR02-004	[redacted]	Manifest Refraction Sphere Right Eye	0.2	RIGHT EYE	BASELINE	[redacted]	-5
ALN-TTR02-004	[redacted]	Manifest Refraction Sphere Right Eye	0.2	RIGHT EYE	MONTH 18	[redacted]	551
ALN-TTR02-004	[redacted]	Manifest Refraction Sphere Left Eye	0.4	LEFT EYE	BASELINE	[redacted]	-6
ALN-TTR02-004	[redacted]	Manifest Refraction Sphere Left Eye	0.7	LEFT EYE	MONTH 18	[redacted]	553
ALN-TTR02-004	[redacted]	Manifest Refraction Sphere Right Eye	0.7	RIGHT EYE	MONTH 18	[redacted]	565
ALN-TTR02-004	[redacted]	Manifest Refraction Sphere Right Eye	0.8	RIGHT EYE	MONTH 18	[redacted]	553
ALN-TTR02-004	[redacted]	Manifest Refraction Cylinder Right Eye	1.05	RIGHT EYE	MONTH 18	[redacted]	570

6. Cup to disc ratios are determined by direct observation. They are not values normally read off a machine. The audit report should clarify the following items.

   Patisiran 0.3 mg/kg
   Subject [redacted] (b) (6)
   STUDY PROCEDURE Dilated Indirect Ophthalmoscopy: Cup-To-Disc Ratio was not performed as the machine was not able to do this procedure [redacted] (b) (6)

   STUDY PROCEDURE For 18 month visit the following procedures were not done: -Visual Acuity: LogMAR Score Left Eye (OS)-Dilated Indirect Ophthalmoscopy: Cup-To-Disc Ratio (Equipment did not allow to obtain the cup-to disc ratio). -Fundus Photography (No equipment available to perform the assessment by that time) [redacted] (b) (6)

7. Visual fields are not appropriately represented by mean deviation. Please submit copies of all visual fields.

Response from Applicant:

Question 1 Response for Study 003 and 004: The study Reference Manual has been added.

Reviewer's Comment: *Acceptable.*

Question 2 Response for Study 003 and 004: The ophthalmologists used their clinical judgement to determine if ocular structures were normal, abnormal/not clinically significant (NCS), or abnormal/clinically significant (CS). For biomicroscopy examinations, no criteria were provided for making this clinical assessment. For indirect ophthalmoscopy examinations, investigators were instructed to classify abnormal findings that "may interfere with study parameters or otherwise confound the data" as CS. Abnormal findings, regardless of clinical significance, were recorded in the electronic data capture (EDC) system for the study and reported in the respective clinical study reports (ALNTTR02-003 Appendices 16.2.8.11, 16.2.8.13, ALN-TTR02-004 Appendices 16.2.8.11,16.2.8.13).

Reviewer's Comment: *The method of describing abnormalities used in the study is not sufficient to allow identification of ocular adverse events or to provide data that can be used to label ocular adverse events potentially caused by the product.*

Question 3 Response for Study 003: In summary, the AE of visual field defect reported during Study 003 was assessed by the Investigator as possibly related to study drug; repeat visual field testing on July 2016 (unscheduled exam performed) and February 2017 (during participation in Study 006) showed stabilization of the visual field tests. Furthermore, ERG testing on July 2016 was normal with no evidence of alteration of rod dysfunction, suggesting that vitamin A deficiency was unlikely to be the etiology of the defect. The AE of decreased visual acuity at night was thought to be due to worsening of a preexisting cataract.

Reviewer's Comment: *Study 006 Week 52/Day 1123 demonstrates stabilization of the right eye visual field, but continued worsening of the left eye visual field. There is agreement that the changes are unlikely to be the result of a vitamin A deficiency, but that does not preclude the changes being in association with the drug product.*

Question 4 Response for Study 003 and 004: In Study 003 and Study 004, the causal relationship of adverse events (AEs) to study drug in the table above was determined by the investigators based on their knowledge of the patient's medical history, the underlying disease (hATTR amyloidosis), and their understanding of the study drug (based on the Investigator's Brochure and protocol). In the overall analysis of the safety profile of patisiran-LNP, Alnylam also evaluated AEs independently of the investigator assessed causality. As noted above, patients with hATTR amyloidosis commonly have ocular findings.

Reviewer's Comment: *The following are reproduced from the applicant's response:*

> "It is also possible that the epiretinal membrane may have been present earlier and may have contributed to the history of decreased vision and other ocular AEs of visual changes."

"The drusen may also be an age-related finding."

"The patient's first dose of patisiran-LNP was on 27Jan2014. On dilated exam (DIO), patient had an epiretinal membrane of the right eye noted at Wk 54 (2/5/2015) that was queried and thought to be missed at baseline. Thus, this finding most likely was a pre-existing condition that may have progressed."

"All events, including the epiretinal membrane, were considered not related or unlikely related by the investigator, perhaps since epiretinal membranes are commonly due to age-related condition of posterior vitreous detachment and the patient had other ocular findings at baseline that could be contributing factors for the reduced visual acuity of the left eye and blurred vision."

These comments and others represent a systematic bias against reporting any ocular event as being associated with the drug product.

Question 5 Response for Study 003 and 004: As described below, Alnylam has completed a thorough review of the potential errors identified by FDA. This was achieved by direct communication with the sites and the review of source documentation. In most of the cases, Alnylam has been able to confirm the data as valid and not erroneous.

For the majority of records identified, the ophthalmologists had applied their standard practice for calculating LogMAR score or assessing cup-to-disc ratio rather than following the Study Reference Manual. In addition, Alnylam has identified some instances of an error in EDC entry or the source worksheet. To assess the impact of the these errors as well as the alternative methods of LogMAR calculation on the summaries reported in the NDA, Alnylam has conducted a series of sensitivity analyses excluding these records.

As identified below, most of the potential errors identified by FDA and confirmed by Alnylam's edit checks were the result of the sites' ophthalmologists utilizing their standard method for visual acuity assessment. In the majority of those cases, although the Study Reference Manual (refer to 5.3.5.2) recommends measuring LogMAR visual acuity using an EDTRS chart, Snellen visual acuity was captured instead, and the ophthalmologist, or site staff, converted the measurement to LogMAR (thus resulting in the logMAR scores not being a multiple of 0.02). Below is a by-study summary of each of the identified sources of potential errors followed by findings for each of the records identified by FDA, as well as those additional records identified by Alnylam in Table 2 (Study 003) and in Table 3 (Study 004). For the remaining 5 of 22 records, the errors are attributed to isolated transcription errors: 4 records (patient [redacted] (b) (6) OS screen/baseline, patient [redacted] (b) (6) OS screen baseline, patient [redacted] (b) (6) OU week 54) had 0.00 checked and a non-zero result (1 or 0.1) was entered, which were then concatenated in the dataset leading to '0.001'(2 cases) or '0.000.1' (2 cases) being displayed. The remaining record (patient [redacted] (b) (6) at Week 108 OD) appears to be due to a transcription error. Our review of the source has confirmed the handwriting is difficult to read.

For 33 of the 35 records, communication with the site and review of the source files revealed that the ophthalmologist (or staff) derived the LogMAR visual acuity score by a method other than what was recommended in Study Reference Manual. As in study 003, sites 037, 050, 053, 060, 061, 062, used EDTRS charts to measure Snellen visual acuity and then converted that value to LogMAR. Site 150

reportedly used an online calculator to derive LogMAR visual acuity. For the remaining 2 of 35 records in this category, isolated errors were identified. Patient [redacted] (b) (6), Month 9, OD, the source states 0.9, whereas 0.99 was entered in EDC.Patient [redacted] (b) (6) Month 9, OD, the EDC entry matches the source worksheet (underlying cause unknown). In addition to the sites listed above, sites 080, 086, and 100 also reported measuring Snellen visual acuity and converting to LogMAR. However, LogMAR records from these sites were all multiples of 0.02.

Sphere or Cylinder Values Not in Quarters of a Diopter
Alnylam obtained and reviewed the majority of source worksheets for this category (14 of 17 records) across the two studies. For Study 003, the Agency identified 3 records in this category in the Information Request. Alnylam identified an additional 1 record via programmed data check, for a total of 4 records. For all 4 records, the EDC entry matches the source worksheet. The underlying reason for the error is unknown.

A total of 13 records (0.4% and 1.9% of cup-to-disc records for patisiran-LNP-treated and placebo-treated patients, respectively) in 7 patients were identified in Study 004. For 7 of 13 records, the EDC entry matches the source worksheet. The underlying reason for the error is unknown. For 3 of 13 records (patients [redacted] (b) (6)), the source worksheets were not sufficiently legible to determine their cause. Therefore, we will consider these to be errors, but unable to be corrected. For the remaining 3 records (patients [redacted] (b) (6) OU, [redacted] (b) (6) OD), the source files have not been received from the site.

Reviewer's Comment: *The failure to follow the procedures identified in the protocol is problematic. The failure to follow procedures in the protocol was identified because values seen in the database raised questions about accurate data collection. It is not known how many other procedures were not followed but not identified because the database values were not unexpected.*

Protocol ALN-TTR2-004 Ocular Adverse Events from Table 14.3.1.2.1 Treatment-Emergent Adverse Events
Events listed are events which occurred in at least 2 patients on the treatment group

Adverse Event	Placebo (N=77)		Patisiran (N=148)	
Eye disorders	20	26%	41	28%
Cataract	5	6%	8	5%
Cataract nuclear	0		2	1%
Cataract subcapsular	0		1	1%
Dry eye	2	3%	7	5%
Eye irritation	0		2	1%
Eyelid ptosis	1	1%	2	1%
Blurred vision	1	1%	4	3%
Visual impairment	0		2	1%
Vitreous floaters	1	1%	3	2%
Vitreous opacity	1	1%	2	1%
Intraocular hematoma	0		2	1%

Reviewer's Comment: *The various types of cataracts should be grouped together to report an accurate percentage. The vitreous floaters and vitreous opacity should be grouped together. Intraocular hematoma is questioned as a correct diagnosis.*

As submitted in this study, dry eye, blurred vision and vitreous floaters should be included as potential adverse events in the product labeling.

[1] If a patient experienced more than 1 event in a given SOC, that patient is counted once for the SOC. If a patient experienced
more than 1 event with a given PT, that patient is counted only once for that PT.
[2] The total number of events for all patients; a patient can be counted more than once if the patient has multiple events.
TEAEs are those with onset during or after the first dose through 28 days following the last dose of study drug. In addition, any event
that was present at baseline but worsened in intensity or was subsequently considered drug-related is considered a TEAE. The Medical Dictionary for Regulatory Activities (MedDRA) version 18.0 is used to code adverse events.

Reviewer Responses to Division's Questions:

1. Please evaluate the clinical study adverse event data and electroretinogram (ERG) data for evidence of ocular toxicity related to vitamin A deficiency.

Reviewer's Comment: *The submitted ERGs have been reviewed. The visual fields have been reviewed. There is no evidence of ocular toxicity related to vitamin A.*

2. Please comment on whether the applicant adequately evaluated for ocular toxicity related to vitamin A deficiency in the clinical studies of patisiran. If applicable, provide recommendations for further evaluation.

Reviewer's Comment: *There are multiple instances of the ophthalmic investigators not following the protocol and the applicant not adequately monitoring the ophthalmologic examinations. As a result, there is very poor reliability of the ocular toxicology findings. However, vitamin A deficiencies in the eye are easily recognized and there is no evidence in the clinical trials of ocular vitamin A deficiencies.*

3. Please comment on the adequacy of the applicant's proposed labeling to reduce the potential risk of ocular toxicity due to vitamin A deficiency.

Reviewer's Comment: *From an ophthalmologic perspective, there is no objection to the applicant's proposed recommendation to supplement with vitamin A, as there were no ocular vitamin A related abnormalities identified in the clinical trials.*

Based primarily on Study 004, the following ocular adverse reactions which may be related to the use of the drug product should be included in the labeling: dry eye, blurred vision and vitreous floaters.

Wiley A. Chambers, M.D.
Supervisory Medical Officer, Ophthalmology

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

WILEY A CHAMBERS
06/18/2018

Office of Clinical Pharmacology Review

NDA	210922
Link to EDR	\\cdsesub1\evsprod\nda209360
Submission Date	12/11/2017
Submission Type	505 (b) (1), Priority Review
Brand Name	ONPATTRO™
Generic Name	Patisiran
Dosage Form and Strength	Sterile, solution for intravenous infusion (10 mg/5 ml, 2 mg/ml patisiran, equivalent to 2.1 mg patisiran sodium salt) in a single- (b) (4) vial
Route of Administration	Intravenous
Proposed Indication	Treatment of adults with hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis)
Applicant	Alnylam Pharmaceuticals, Inc.
Associated IND	117395
OCP Review Team	Venkateswaran Chithambarampillai, MS(Pharm), PhD; Venkatesh Atul Bhattaram, PhD; Hobart Rogers, PharmD, PhD; Theingi Thway, PhD; Christian Grimstein, PhD; Kevin Krudys, PhD; Sreedharan Sabarinath, PhD.
OCP Final Signatory	Mehul Mehta, PhD.

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1. EXECUTIVE SUMMARY

Alnylam Pharmaceuticals, Inc is seeking approval for patisiran (ONPATTRO™) for the treatment of adults with hereditary transthyretin-mediated amyloidosis via 505(b)(1) pathway. hATTR is a rare and fatal disease caused by mutations of genes that code for transthyretin (TTR) protein. TTR is a tetrameric protein primarily produced in hepatocytes. Genetic mutations in the *TTR* gene can act to thermodynamically destabilize the tetrameric TTR protein into monomeric units which can then misfold and aggregate as amyloid deposits in various tissues including nerves, heart, kidney, liver, gastro-intestinal tract and other tissues, and cause progressive cellular degeneration and death. Patisiran consists of double-stranded (ds) small interfering RNA (siRNA, 21-mers) encapsulated in lipid nanoparticles (LNP). LNP enable targeted delivery of siRNA into hepatocytes. Patisiran binds to target sequence in the 3' untranslated region of TTR mRNA, thus degrading both wild-type and mutant TTR.

The application relies on safety and efficacy of patisiran from a multi-center, randomized, double-blind, placebo-controlled, pivotal Phase 3 clinical trial (APOLLO; Study ALN-TTR02-004) of 18 months duration in patients with hATTR with polyneuropathy (hATTR-PN) as the basis for approval. The trial evaluated the efficacy and safety of patisiran at one dose level of 0.3 mg/kg IV infusion over approximately 80 min once every three weeks. For patients weighing ≥100 kg, the dose was capped to 30 mg. This study demonstrated a statistically significant benefit in favor of patisiran treatment compared to placebo in change in modified neuronal impairment score (mNIS+7, primary efficacy endpoint) from baseline to month 18. Patisiran also showed statistically significant improvement on all secondary endpoints including, neuropathy symptom specific quality of life (Norfolk QoL-DN), motor strength (NIS-W), disability (R-ODS), gait speed (10-MWT), nutritional status (mBMI) and autonomic symptoms (COMPASS 31) at 18 months.

The primary objectives of this review are:

1) to evaluate the appropriateness of the proposed dose and dosing regimen of patisiran,
2) to assess the effect of immunogenicity on patisiran pharmacokinetics, pharmacodynamics, and efficacy, and
3) to evaluate the adequacy of labeling statements based on population pharmacokinetic analyses.

1.1 Recommendations

The Office of Clinical Pharmacology has reviewed the information contained in this NDA and recommends approval from a clinical pharmacology perspective. The review focus with specific recommendations and comments are summarized below.

Review Summary	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	Primary evidence of effectiveness was established from a single pivotal randomized, placebo-controlled Phase 3 study in patients with hATTR-PN.
General dosing instructions	The proposed dosing regimen of patisiran is 0.3 mg/kg IV infusion over 80 min once every 3 weeks. For patients weighing ≥100 kg, the dose is capped to 30 mg.
Dosing in patient subgroups (intrinsic and extrinsic factors)	No dose adjustments are required. Hepatic/renal impairment is not expected to affect patisiran exposures. Drug interaction liability with patisiran is considered low.
Labeling	The labeling concepts proposed by the Applicant are generally adequate.
Bridge between the to-be-marketed and clinical trial formulations	The to-be-marketed formulation is the same as the one used in the pivotal efficacy study.

1.2 Post-Marketing Requirements and Commitments

None

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

Mechanism of Action: Patisiran contains ds siRNA targeting TTR mRNA encapsulated in LNP. Following IV infusion, LNP delivers siRNA into hepatocytes. In the cytoplasm of hepatocytes, the siRNA integrates into the RNA-induced silencing complex (RISC). This integration cleaves the ds siRNA into a single strand with RISC complex. The antisense strand then recognizes and binds to the complementary sequence in the 3'-UTR of the TTR mRNA and cleaves it by the endonuclease argonaute 2, thus degrading both wild-type and mutant TTR production.

Pharmacokinetics:

Following IV infusion, patisiran exposures [maximum plasma concentration (C_{max}) and area under the plasma concentration-time curve (AUC)] increased in a linear and dose-proportional manner in healthy subjects.

Distribution: Patisiran is primarily distributed in the liver. Plasma protein binding is low (<2.1%).

Metabolism: Patisiran is mainly metabolized by nucleases to shorter nucleotides of varying length.

Excretion: The mean terminal elimination half-life of patisiran is approximately 3 days. Less than 1% of administered patisiran is excreted unchanged in the urine.

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

The recommended dose of patisiran in adults is 0.3 mg/kg IV infusion over 80 min once every 3 weeks. For patients weighing ≥100 kg, the recommended dose is 30 mg. This is the same dosing regimen studied in pivotal efficacy trial.

2.2.2 Therapeutic individualization

No therapeutic individualization is required for patisiran based on intrinsic or extrinsic factors. Patisiran is not a substrate, inhibitor, or inducer of major CYPs or transporters. Intrinsic factors including hepatic/renal impairment are not expected to significantly affect patisiran exposures.

2.3 Outstanding Issues

None.

2.4 Summary of Labeling Recommendations

The labeling concepts proposed by the Applicant are generally adequate. The Office of Clinical Pharmacology recommends removing section [redacted] (b) (4) and include the information currently described in [redacted] (b) (4) such as "patisiran has not been studied in patients with prior liver transplant", in Section 12.3 Pharmacokinetics.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

Patisiran drug product (patisiran-LNP) consists of ds siRNA (ALN-18328; 21 oligonucleotides with 2′-O-methylation at cytidine and uridine residues, molecular weight: 13424 Da) encapsulated in LNP. This LNP formulation facilitates delivering of the siRNA into human hepatocytes. LNP has two novel lipid excipients, DLin- MC3-DMA and PEG_{2000}-C-DMG. DLin- MC3-DMA is essential for [redacted] (b) (4) PEG_{2000}-C-DMG is [redacted] (b) (4) In the circulation, PEG_{2000}-C-DMG dissociates from the surface of LNP which allows binding of circulating apoE lipoprotein to the surface of LNP and thus facilitate the uptake of patisiran-LNP into human hepatocytes through apoE receptor mediated endocytosis. Patisiran siRNA is an inhibitor of the production of both wild-type and mutant TTR proteins through degradation of TTR mRNA. It is developed as a solution for IV infusion use. Currently, no drug is approved for the treatment of hATTR-PN in the U.S.

On June 14, 2012, patisiran received orphan designation from the Agency for the treatment of hATTR-PN. On April 29, 2013, IND117395 was submitted along with a request for breakthrough therapy designation (BTD). On August 7, 2013, BTD request was denied due to

inadequate demonstration of substantial improvement over existing therapies on one or more clinically significant endpoints. On September 23, 2013, an End-of-Phase 2 meeting was held to discuss the overall development plan to support the new drug application for patisiran. On October 31, 2013, patisiran received fast-track designation. On October 19, 2017, the Agency agreed with the Sponsor for the rolling submission of patisiran NDA. On November 13, 2017, the Agency held a pre-NDA meeting with the Sponsor to discuss the content and format of patisiran NDA for the treatment of patients with hATTR-PN. The Sponsor also confirmed that the clinical trial formulation of patisiran will be the same as the to-be-marketed formulation. On November 17, 2017, patisiran received BTD.

The clinical studies that were submitted as part of the patisiran NDA included two phase 1 single ascending dose studies (ALN-TTR02-001 and ALN-TTR02-005) in healthy volunteers, a phase 2 multiple ascending dose study (ALN-TTR02-002) and its extension study (ALN-TTR02-003) and a phase 3 study (ALN-TTR02-004) in patients with hATTR-PN.

3.2 General Pharmacology and Pharmacokinetic Characteristics

Pharmacology	
Mechanism of Action	Patisiran siRNA binds to RISC complex in the cytoplasm of human hepatocytes which results in cleavage of ds siRNA to a single strand within the RISC complex. Following hybridization of this single stranded siRNA-RISC complex with target TTR mRNA, the RISC complex uses the endonuclease argonaute 2 to cleave the TTR mRNA and thus inhibits the translation of mRNA into TTR protein.
QT Prolongation	Thorough QT study (TQT) was waived due to a low likelihood of direct ion channel interactions. There is no evidence from nonclinical or clinical data to suggest that patisiran has the potential to delay ventricular repolarization.
General Information	
Bioanalysis	Plasma concentrations of patisiran siRNA were measured using a validated liquid chromatography with fluorescent detection method. Plasma concentrations of the lipid components, DLin-MC3-DMA, and PEG_{2000}-C-DMG were quantitated using a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) method. Serum TTR concentrations were measured using a validated ELISA method. Details are described in section 4.1.
Healthy Volunteers vs. Patients	Plasma patisiran concentrations are similar between hATTR-PN patients and healthy subjects.
Dose Proportionality	The PK is linear and dose-proportional over the dose range of 0.01-0.3 mg/kg dose.
Variability	Inter-individual variability in plasma C_{max} of patisiran (%CV) ranges from 30-38% and $AUC_{0\text{-}last}$ (%CV) for patisiran ranges from 84% to 110%.

Immunogenicity	Anti-drug antibodies (ADAs) specific to PEG_{2000}-C-DMG were detected using a validated ELISA. In the placebo-controlled studies, approximately 4.1% of patisiran treated patients (6/148) showed detectable ADAs. However, the ADA titers were low and their appearance was transient. ADA status did not seem to influence the clinical efficacy, pharmacokinetic or pharmacodynamic profiles of patisiran. However, the number of subjects with ADA positive test were very limited to rule out the impact of ADA effects. For more details, refer to section 4.5.
Absorption	
T_{max}	Patisiran is administered as IV infusion and it reaches maximum plasma concentration at the end of infusion period.
Distribution	
Volume of Distribution	The apparent volume of distribution at steady state is (b) (4) L/kg.
Protein Binding	<2.1%
Substrate/Inhibitor of transporter systems	Not a substrate or inhibitor for BCRP, MDR1, BSEP, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2-K *in vitro.*
Elimination	
Terminal Elimination half-life	The elimination half-life ranges from (b) (4) days.
Metabolism	
Primary Metabolizing enzymes	Endonucleases and exonucleases
Inhibitor/Inducer	Not an inhibitor for CYP3A4/5, CYP1A2, CYP2C9, CYP2C19 and CYP2D6 or an inducer for CYP3A, CYP1A2 and CYP2B6.
Excretion	
Primary excretion pathways	Excreted by the kidney as chain-shortened oligonucleotides, which are not considered pharmacologically active. Less than 1% of the administered dose of patisiran is excreted unchanged into urine.

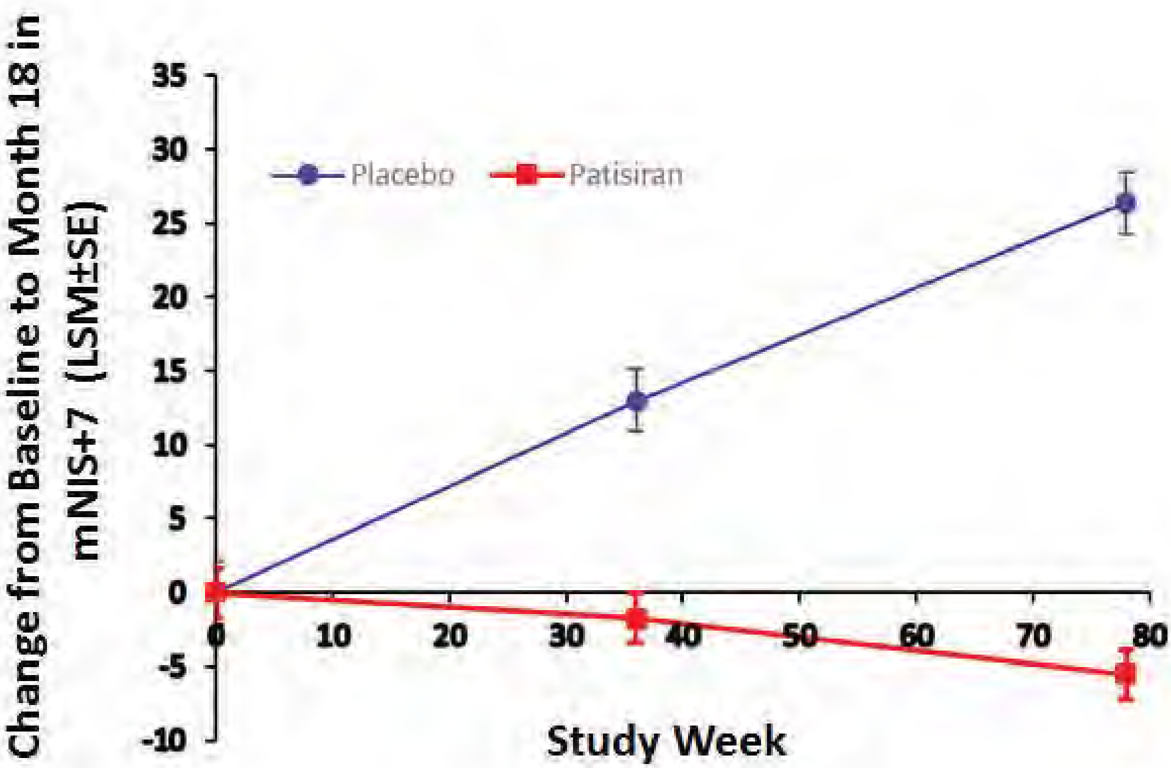
3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

The primary evidence of the efficacy of patisiran was demonstrated from a pivotal phase 3, placebo-controlled, randomized, double-blind, multi-center study (APPOLLO; Study ALN-TTR02-004) in patients with hATTR-PN. The study was conducted in 44 centers worldwide. A total of 225 patients were enrolled in this study and were randomized (2:1) to receive either patisiran at 0.3 mg/kg IV infusion over approximately 70 min or longer every 21 days (Q3W)

[N=148] or placebo (normal saline) [N=77] over 18 months. The primary efficacy endpoint was the comparison of change from baseline to month 18 in mNIS+7 score between patisiran and placebo. The study demonstrated a statistically significant improvement in neuropathy for patients treated with patisiran [least square mean (LSM) difference in ΔmNIS+7 score: -33.99 points] compared to placebo at 18 months (Figure 1). These results indicate that the study met the primary efficacy endpoint. Patisiran also demonstrated a statistically significant improvement on all secondary endpoints including, neuropathy symptom specific quality of life (Norfolk QoL-DN), motor strength (NIS-W), disability (R-ODS), gait speed (10-MWT), nutritional status (mBMI) and autonomic symptoms (COMPASS 31) at 18 months. Refer to the statistical review by the Office of Biostatistics for details regarding the statistical significance.

Figure 1. LSM change from baseline to month 18 in mNIS+7 score between patisiran and placebo in patients with hATTR-PN

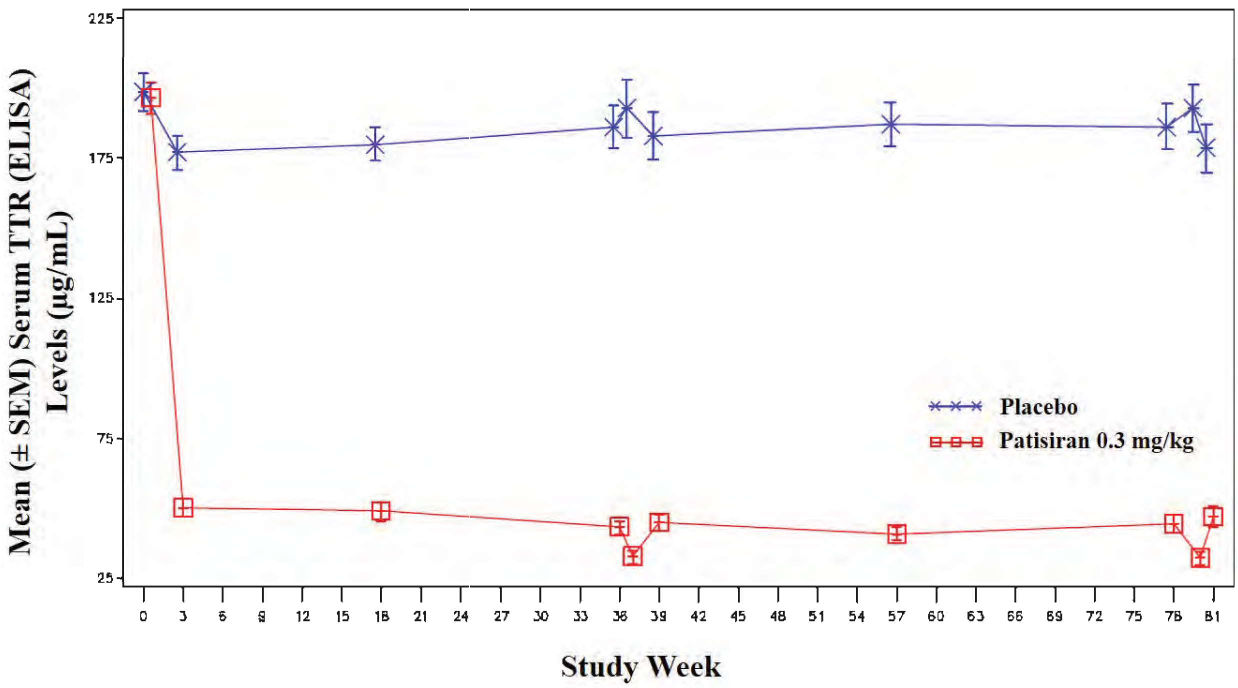


Source: Study ALN-TTR02-004; Module 5.3.5.1; section 14 Tables, Figures and Graphs; Table 14.2.1.1.1

Pharmacodynamic effect on serum TTR levels:
Patisiran showed a sustained suppression of serum TTR protein levels over 18 months in the pivotal phase 3 study (Figure 2). Patisiran achieved an average of 78% reduction in serum TTR protein compared to 6% reduction with placebo. Maximum reduction in serum TTR levels was observed following administration of the first dose of patisiran. Based on the single ascending

dose study of patisiran in healthy volunteers, it was expected that the maximum reduction in serum TTR could be observed as early as 7-10 days after initiation of patisiran treatment (please refer to section 3.3.2). These findings are consistent with the mechanism of action of patisiran.

Figure 2. Average (±SEM) reduction of serum TTR protein levels over time between patisiran and placebo in patients with hATTR-PN



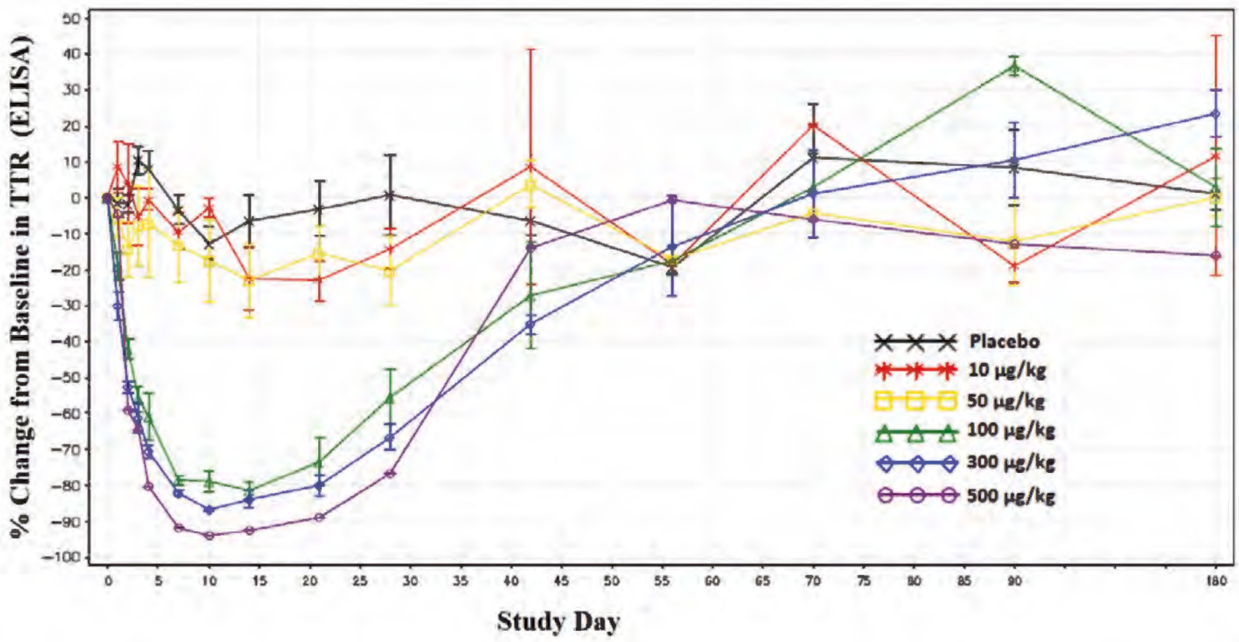
Source: Study ALN-TTR02-004; Module 5.3.5.1; section 14 Tables, Figures and Graphs; Figure 14.2.3.1

3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes, the proposed dose and dosing regimen for patisiran is appropriate for the intended patient population. Patisiran showed a dose-dependent reduction in serum TTR protein from 50 µg/kg to 500 µg/kg in healthy volunteers. Serum TTR reduction was reached near to the maximum level at 300 µg/kg dose (Figure 3). Subsequently, patisiran was evaluated at 0.3 mg/kg dose level in the registration trial (APOLLO).

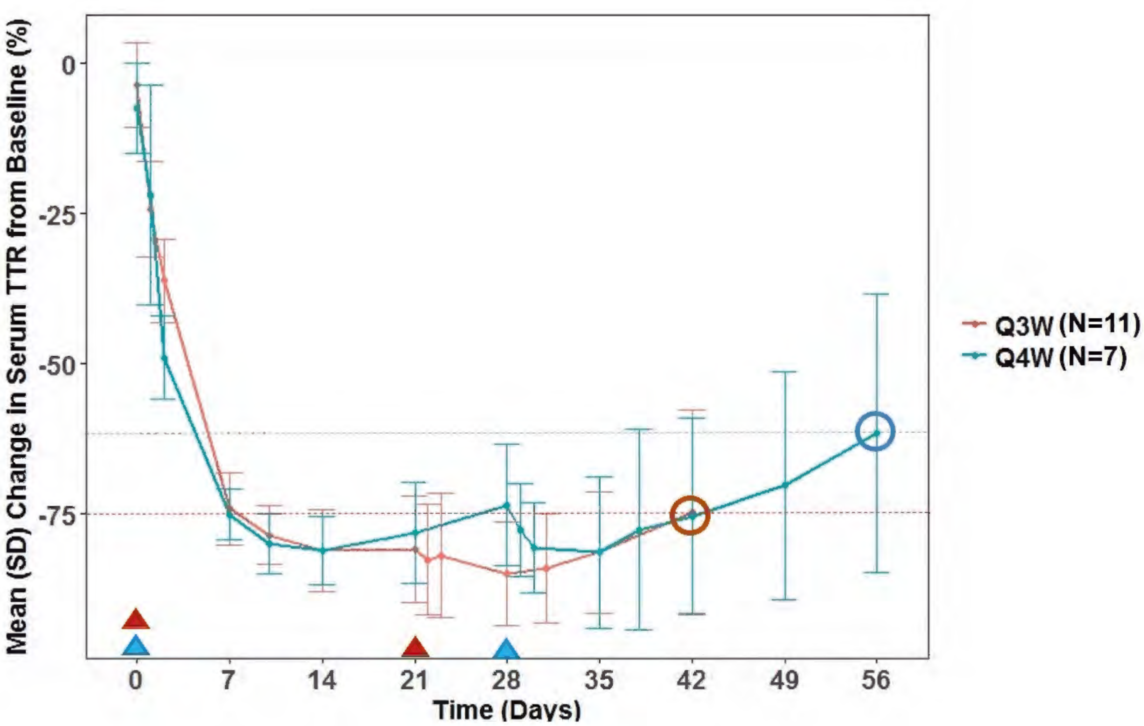
Following single IV infusion, the time to achieve maximum reduction of serum TTR protein level ranged from day 7 to day 10, and such reduction was maintained over day 21. To select an optimal dosing regimen, the Applicant conducted a phase 2 trial to compare the extent of serum TTR reduction at the end of second dosing interval between three weekly regimen (Q3W) and four weekly regimen (Q4W) in patients with hATTR. Patients received two doses of patisiran on day 0 and either day 21 (Q3W) or day 28 (Q4W). Q3W regimen showed a sustained suppression of serum TTR (75%) compared to Q4W regimen (63%) [Figure 4]. Therefore, Q3W regimen was selected for the registration trial (APOLLO).

Figure 3. Average (±SEM) reduction in serum TTR protein levels over time between patisiran at various doses and placebo in patients with hATTR-PN



Source: Study Report ALN-TTR02-001; Module 5.3.3.1; Figure 14.2.3.1

Figure 4. Average (±SD) reduction in serum TTR protein levels over time after administration of 0.3 mg/kg patisiran either three weekly interval or four weekly interval in patients with hATTR-PN



Filled triangles represent the day of 0.3 mg/kg patisiran administration for Q3W and Q4W. Open circles represent the end of dosing interval following second dose administration.

Source: Population PK Study Reports, ALNY-CSC-122PKPD, alnpkpddataset

Patisiran at dosage regimen of 0.3 mg/kg Q3W demonstrated statistically significant improvement in neuropathy in patients with hATTR-PN. The serum TTR protein levels remained suppressed throughout 18 months of treatment in pivotal phase 3 study (please refer to section 3.3.1). Therefore, the Applicant's proposed dose and dosing regimen of patisiran for the treatment of patients with hATTR-PN is acceptable.

3.3.3 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors?

No. Age, gender, race, renal or hepatic impairment did not affect the systemic exposures of patisiran. There was a trend towards increasing exposures to patisiran with increasing body weight (please refer to section 4.3). In patients weighing over 100 kg, the exposures observed following administration of 0.3 mg/kg dosing is comparable to the exposures normalized to 30 mg equivalent dosing. Therefore, the proposed 0.3 mg/kg Q3W dosing for patients weighing up to 100 kg and 30 mg Q3W dosing for patients weighing over 100 kg are acceptable. Given that patisiran is primarily metabolized by nucleases, the intrinsic factors are not expected to alter the systemic exposures to patisiran. Therefore, alternate dosing regimen of patisiran is not required for subpopulations based on intrinsic factors.

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

No. Because patisiran is administered intravenously, a food-drug interaction is not expected. In vitro drug interaction studies suggest that patisiran is not a substrate and/or inhibitor for major CYP enzymes and transporters. Therefore, drug-drug interaction liability of patisiran is anticipated to be minimal.

4. APPENDICES

This section includes information on – (a) bioanalytical method validation and performance supporting all pharmacokinetic studies, and (b) brief description of study design and detailed pharmacokinetic and pharmacodynamic results from the studies submitted in this application.

4.1 Summary of Bioanalytical Method Validation and Performance

Plasma concentrations of patisiran siRNA (ALN-18328) was measured by validated liquid chromatography with fluorescent detection (TSLR11-050). Lipid components, DLin- MC3-DMA and PEG_{2000}-C-DMG were quantitated by validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods (TSLR10-041L, TSLR10-041H and TSLR10-049). All the methods were adequate. The summary of method performances are shown in Table 1 below. Additionally, it was found that:
The precision and accuracy values (

- Table 1) of at least two-thirds of the overall QC samples were equal to or better than 15% (20% at the LLOQ) from the supporting bioanalytical reports.
- Patisiran siRNA, DLin- MC3-DMA and PEG_{2000}-C-DMG were found to be stable in plasma after at least three freeze-thaw cycles at -70˚ C, at room temperature in whole blood over atleast 2 h (short-term), at -20˚ C storage in human plasma over atleast 54 days for (long term), bench-top stability in human plasma at 1 to 8˚ C for at least 6 h and processed sample stability at 1 to 8˚ C for at least 167 h.
- The QC sample accounting for dilution showed an acceptable precision (<3%) and bias (<9%). No carryover effects were observed. However, an occasion carry over effect for siRNA was observed in run 4 but not in any of the previous 3 runs. Therefore, it was considered that due to syringe issue rather quantitation method.
- More than two-thirds of the incurred sample reanalysis (ISR) fell within 20% deviation.

Table 1. Summary of bioanalytical methods and validation procedures for patisiran siRNA, DLin- MC3-DMA and PEG_{2000}-C-DMG

Bioanalytical site	Analytical method	Analyte	Sample volume	Analytical range (ng/ml)	Precision (CV %)	Accuracy (% Diff)
[redacted] (b) (4)	LC-Fluorescent detection	ALN-18328	30 µl	1-250	≤10.6%	-12.7-8.0
	LC-MS/MS (Low range)	DLin- MC3-DMA	50 µl	0.5-100	≤5.0%	-8.0-4.4
	LC-MS/MS (High range)	DLin- MC3-DMA	50 µl	50-50000	≤8.8%	-7.5-8.3
	LC-MS/MS	PEG_{2000}-C-DMG	100 µl	5-5000	≤8.3%	-12.0-5.0

Source: Module 5.3.1.4; Bioanalytical Reports from all studies

Serum concentrations of TTR protein were measured using a validated ELISA (Analytical procedures AP.302417.TTR.01 and AP.302417.TTR.02) in which a rabbit polyclonal anti-TTR antibody was used as capture reagent, and sheep polyclonal anti-TTR antibodies followed by alkaline phosphatase-labeled donkey anti-sheep antibody were used as detection reagents (Validation report # 302417). Standard calibrators were prepared by spiking TTR in 1X power block buffer. Method validation and analysis for measurement of serum TTR for the studies were performed at [redacted] (b) (4)). Study samples and QCs were diluted up to 1/16000 in power block buffer prior to analysis. The validation procedures are briefly summarized in Table 2.

Table 2. Summary of bioanalytical methods and validation procedures for serum TTR measurement

TTR method review summary	There were a few minor method issues; non-reliable LLOQ, and potential hook effect. Serum TTR concentrations <2.04 ng/mL were excluded from the review analysis. Risk of potential hook effect was mitigated by reporting concentrations up to 43.75 ng/mL.
Material for calibration	Prealbumin from human plasma (Sigma-Aldrich Catalog P1742, Lot 058K1395)

curve & source			
Validated assay range	1.13 (LLOQ) – 69.44 (ULOQ) ng/mL in 1X power block buffer		
Source of reagents	Rabbit polyclonal anti-TTR antibody (Abcam, Catalog # ab16006) Sheep polyclonal anti-TTR antibody (Abcam, Catalog # ab9015) Alkaline phosphatase-labeled donkey anti-sheep antibody (Sigma, Catalog # A5187)		
Regression model & weighting	5 parameter logistic auto-estimate with weighting factor 1		
Validation parameters	**Method validation summary**		**Acceptability**
Standard calibrator performance during accuracy & precision	Number of standard levels including LLOQ to ULOQ	7	Yes
	Cumulative accuracy (%bias) in standard calibrators	-4.1 to 2.5%	Yes
	Cumulative precision (%CV)	≤ 6.6%	Yes
QCs performance during accuracy & precision	Cumulative accuracy (%bias) in 5 spiked QCs	-5.4 to 4.9%	Yes
	Inter-batch precision (%CV) 5 spiked QCs Inter-batch precision (%CV) endogenous QCs	≤ 17.7% ≤ 11.0%	Yes
	Total error (TE) in percent	≤ 14.1% ≤ 23.1% (LLOQ)	Yes
Endogenous TTR level	Ranged from 203.2 to 372.16 mcg/mL in 10 lots of human serum		NA
Selectivity	10 serum lots tested were diluted 1/16000 in power block buffer and spiked at 1.13 ng/mL of TTR. At least 9 lots within 100.1-120.5 % from expected concentrations (endogenous plus spiked concentrations).		Yes
Interference	No interference observed with DLinDMA, PEG-C-DMG, DLin-MC3-DMA or PEG-C-DMA		Yes
Hemolysis effect	Not tested		NA
Lipemic effect	Not tested		NA
Dilution linearity & hook effect	See parallelism for dilution. Potential assay hook effect may exist near ULOQ		No, risk mitigated
Parallelism	Tested with 5 serum lots at dilutions ranging from 4,000 to 32,000		Yes
Bench-top/process stability	Stable at room temperature for 14 hrs or at 4°C for 24 hours in human serum		Yes
Freeze-Thaw stability	Up to 7 cycles		Yes
Long-term storage	At nominal -80°C for 733 days and at nominal -20°C for 4 weeks		Yes
Blood collection stability	Tested with 3 lots of blood. Stable for 8 hrs at room temperature and for 1 hr at 30 °C		
Method performance in study ALN-TTR02-002			
Assay passing rate	• 26 out of 28 runs (93%) met the method acceptance criteria.		Yes
Standard curve performance (2.04 -69.44 ng/mL)	• Cumulative bias range: -0.4 to 2.1% • Cumulative precision: ≤ 7 %CV		Yes
QC performance	• Cumulative bias range: -5.5 to -3.1% • Cumulative precision: ≤11 %CV • TE: ≤ 16%		Yes

Study sample analysis/ stability	593 samples analyzed within established long term stability.	
	Method performance in study ALN-TTR02-004	
Assay passing rate	• 87 out of 99 runs (87.9%) met the method acceptance criteria.	Yes
Standard curve performance (2.04 -69.44 ng/mL)	• Cumulative bias range: -2.0 to 2.4% • Cumulative precision: ≤ 12%CV	Yes
QC performance	• Cumulative bias range: -5.4 to -1.2% • Cumulative precision: ≤ 12%CV • TE: ≤ 17%	Yes
Study sample analysis note	16 samples with concentrations <2.04 ng/mL were excluded from analysis. 2311 samples analyzed within established long term stability.	

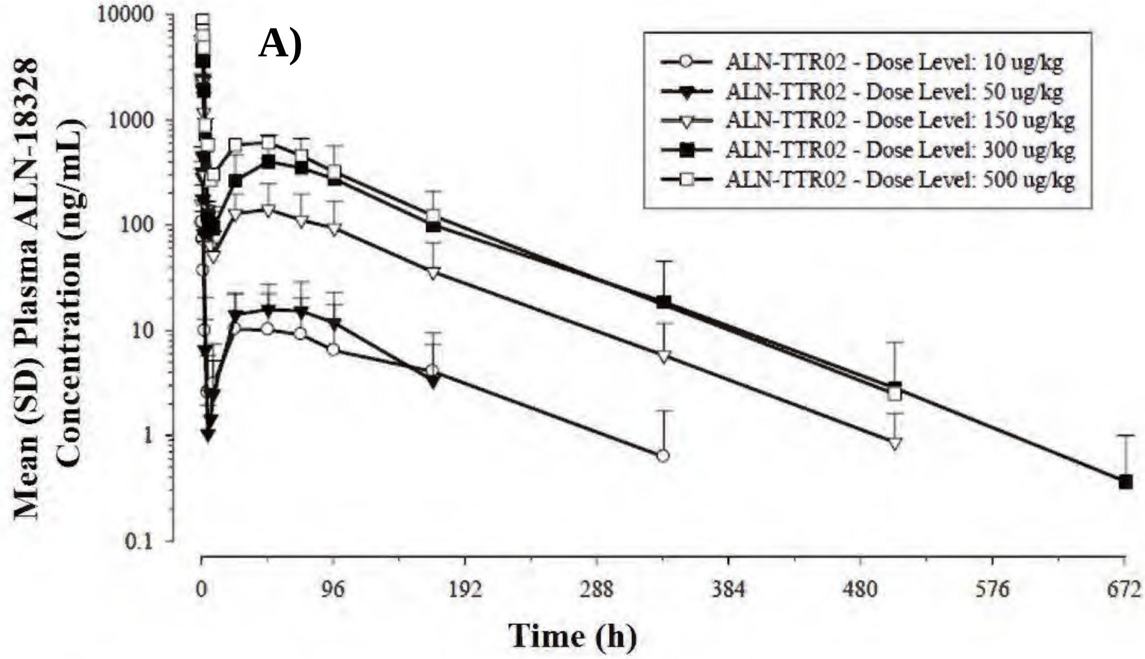
Reviewer comment: The bioanalytical methods for patisiran siRNA, DLin- MC3-DMA and PEG$_{2000}$-C-DMG satisfy the criteria for 'method validation' and 'application to routine analysis' set by the 'Guidance for Industry: Bioanalytical Method Development', and is acceptable. The method of quantitation of serum TTR protein and the validation procedures are also adequate to support clinical sample analysis and the use of TTR as a PD marker.

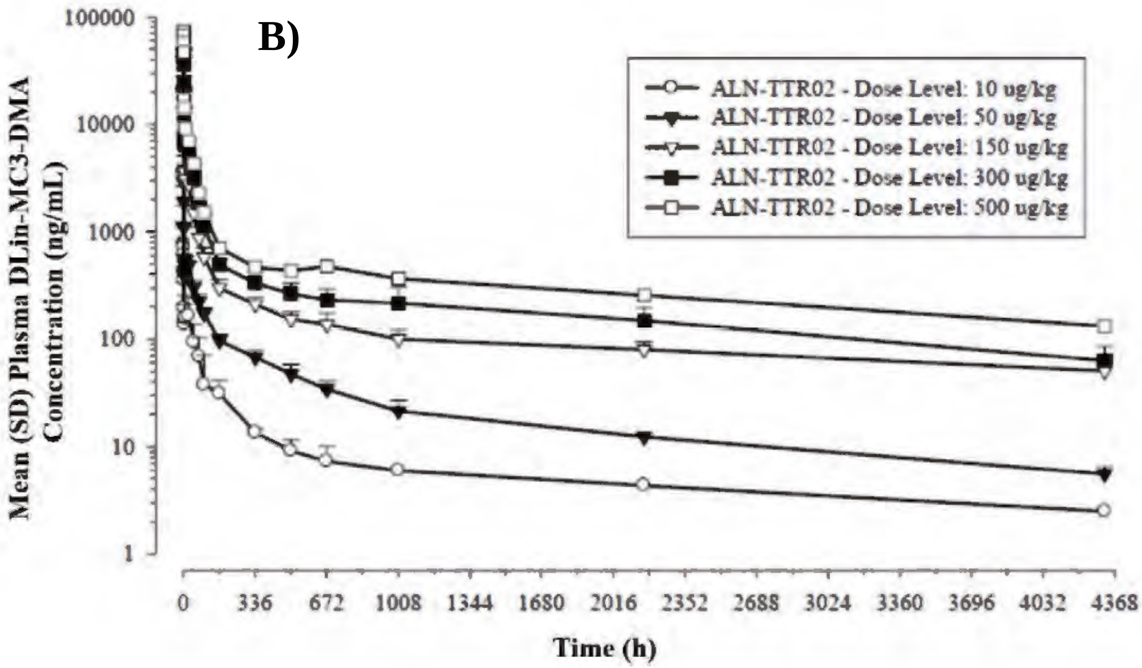
4.2 Clinical PK and/or PD Assessments

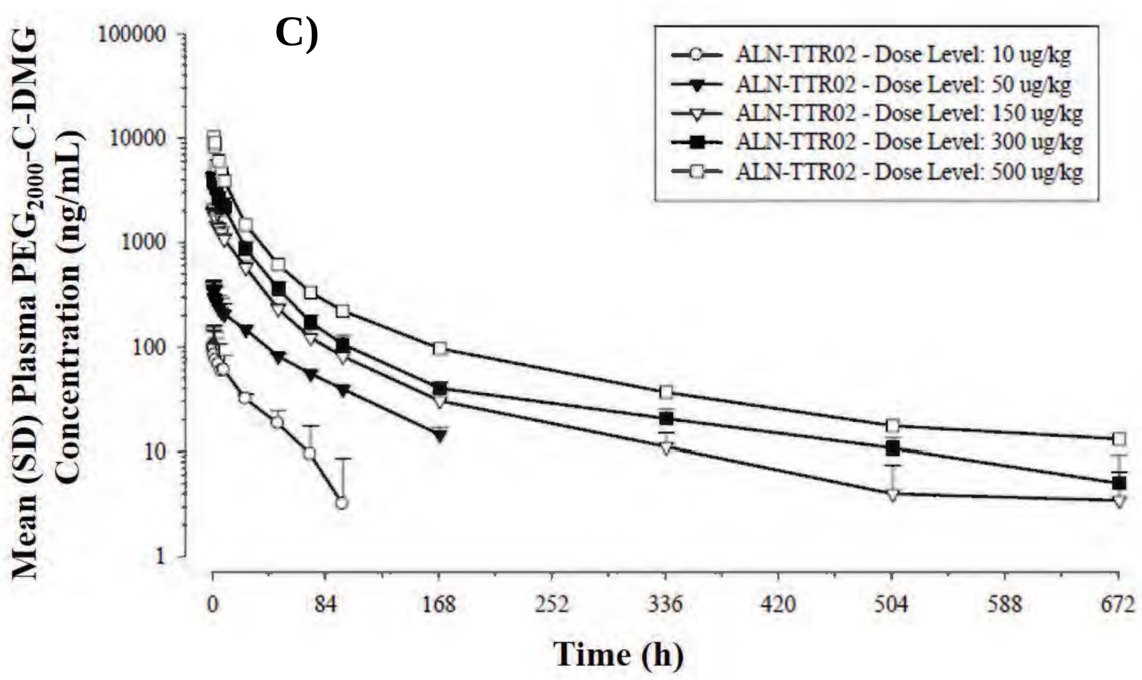
Pharmacokinetics:

Pharmacokinetics of patisiran siRNA (ALN-18328) and lipid components of LNP, DLin- MC3-DMA and PEG$_{2000}$-C-DMG were evaluated in two phase 1 single ascending dose escalation studies (ALN-TTR02-001 and ALN-TTR02-005) in healthy volunteers and a phase 2 multiple ascending dose escalation study (ALN-TTR02-002) and its open label extension study (ALN-TTR02-003) in patients with hATTR following 1 h IV infusion. The doses evaluated in study ALN-TTR02-001 include 10, 50, 150, 300 and 500 µg/kg. A total of 17 subjects were enrolled. Four subjects (3 patisiran: 1 placebo) were enrolled in each dose cohort except in 500 µg/kg dose cohort which enrolled only one subject. Average plasma concentration-time profiles and PK parameters of patisiran siRNA, DLin-MC3-DMA and PEG$_{2000}$-C-DMG in healthy volunteers were shown in Figure 5 and Table 3, respectively.

Figure 5. Plasma concentration-time profiles of patisiran siRNA (A), DLin- MC3-DMA (B) and PEG_{2000}-C-DMG (C) in healthy volunteers.







Source: Module 2.7.2. Summary of Clinical Pharmacology Studies; Figure 6

Patisiran siRNA exerted a biphasic plasma concentration-time profile (Figure 5). The initial rapid decline followed by a secondary rise and linear disappearance in plasma concentrations of siRNA following IV infusion. The initial decline might be possibly due to the rapid distribution of siRNA from the circulation into the liver. Subsequently, a secondary rise in plasma concentration of siRNA may be associated with redistribution of siRNA from the liver to the circulation. The terminal $t_{1/2}$ of siRNA was approximately 2-3 days (Table 3). Less than 1% of the administered dose of patisiran siRNA was excreted into the urine which suggests that renal route of elimination of siRNA contributes to a minor extent for its overall elimination.

Unbound ALN-18328 concentration was also measured using plasma ultrafiltrate using a validated liquid chromatography with fluorescent detection method (TSLR11-043; precision: ≤8.5% and accuracy: -8.0 to 9.0%) following administration of 0.3 mg/kg patisiran-LNP. The mean C_{max} of free ALN-18328 was 0.117 μg/mL and the concentration measured at approximately 48 h post-infusion was 0.000497 μg/mL. At 48 h post-infusion, the unbound concentration of ALN-18328 accounts less than 3% of LNP-associated siRNA and less than 0.1% of the corresponding total siRNA. This low fraction of free siRNA in circulation suggests that the stability of patisiran-LNP in the circulation and the majority of siRNA is within the LNP formulation. Both DLin- MC3-DMA and PEG_{2000}-C-DMG exhibited a typical concentration-time profiles (Figure 5) in the circulation with the terminal $t_{1/2}$ of approximately 78 days and 8 days, respectively.

Table 3. PK parameters of patisiran siRNA (A), DLin- MC3-DMA (B) and PEG_{2000}-C-DMG (C) in healthy volunteers.

A) PK parameters of patisiran siRNA:

PK Parameter	0.01 mg/kg N=3	0.05 mg/kg N=3	0.15 mg/kg N=3	0.3 mg/kg N=3	0.5 mg/kg N=1
C_{max}, µg/mL	0.119 (59.6)	0.450 (23.0)	2.47 (37.9)	5.49 (14.3)	8.79 (NC)
$AUC_{0\text{-last}}$, µg•h/mL	1.64 (137.0)	2.18 (72.7)	23.1 (63.6)	61.5 (81.0)	93.8 (NC)
$AUC_{0\text{-}\infty}$, µg•h/mL	2.65 (NC)	2.47 (71.5)	23.3 (62.8)	61.7 (80.7)	94.0 (NC)
Second phase %AUC[a], %	NC	78.3	80.9	85.8	74.9
$t_{max}{}^{b}$, h	1.08 (1.00-1.17)	1.00 (1.00-1.00)	1.08 (1.08-1.17)	1.00 (1.00-1.00)	2.20 (2.20-2.20)
C_{max2}, µg/mL	0.0154 (NC)	0.0189 (67.0)	0.153 (65.0)	0.412 (76.4)	0.606 (NC)
$t_{1/2\alpha}$, h	0.709 (NC)	0.398 (40.7)	2.32 (8.9)	1.29 (58.7)	1.68 (NC)
$t_{1/2\beta}$, d	2.96 (NC)	2.10 (17.7)	2.37 (23.9)	2.18 (14.9)	2.44 (NC)
$V_{ss}{}^{c}$, L/kg	0.706 (NC)	2.10 (77.9)	0.553 (38.5)	0.552 (70.2)	0.342 (NC)
CL^{c}, mL/h/kg	6.76 (NC)	31.7 (83.4)	9.94 (87.4)	9.35 (100.3)	5.32 (NC)
CL_R, mL/h/kg	NA	0.0271 (NC)	0.0279 (84.7)	0.00547 (136.6)	NC (NC)
Urine $f_e{}^{c}$, %	NC (NC)	0.0332 (NC)	0.167 (17.7)	0.0911 (160)	NC (NC)

Values indicate mean (%CV). NC: Not calculated

B) PK parameters of DLin- MC3-DMA:

PK Parameter	0.01 mg/kg N=3	0.05 mg/kg N=3	0.15 mg/kg N=3	0.3 mg/kg N=3	0.5 mg/kg N=1
C_{max}, µg/mL	0.803 (56.2)	3.14 (19.5)	19.9 (15.2)	45.2 (17.4)	73.3 (NC)
$AUC_{0\text{-last}}$, µg•h/mL	30.9 (56.3)	123 (14.6)	616 (21.2)	1127 (28.3)	1898 (NC)
$AUC_{0\text{-}\infty}$, µg•h/mL	47.9 (NC)	136 (14.4)	860 (24.5)	1297 (28.6)	2327 (NC)
Second phase %AUC[a], %	95.1	96.2	92.0	91.2	NC
$t_{max}{}^{b}$, h	1.00 (1.00-1.08)	1.00 (1.00-1.00)	1.08 (1.00-1.17)	1.10 (1.00-1.17)	2.20 (2.20-2.20)
C_{max2}, µg/mL	0.169 (52.2)	0.544 (8.9)	2.63 (NC)	8.56 (NC)	NC (NC)
$t_{1/2\alpha}$, h	1.81 (NC)	0.620 (NC)	3.35 (NC)	2.05 (NC)	NC (NC)
$t_{1/2\beta}$, d	106 (NC)	70.9 (4.6)	138 (9.7)	78.3 (17.9)	93.8 (NC)
$V_{ss}{}^{c}$, L/kg	3.70 (NC)	3.77 (16.8)	4.31 (16.3)	3.05 (37.7)	3.50 (NC)
CL^{c}, mL/h/kg	1.68 (NC)	2.70 (15.4)	1.32 (28.2)	1.79 (32.9)	1.56 (NC)
DMBA urine $f_e{}^{e}$, %	NA[d]	5.26	4.18	5.48	3.17

Values indicate mean (%CV). NC: Not calculated

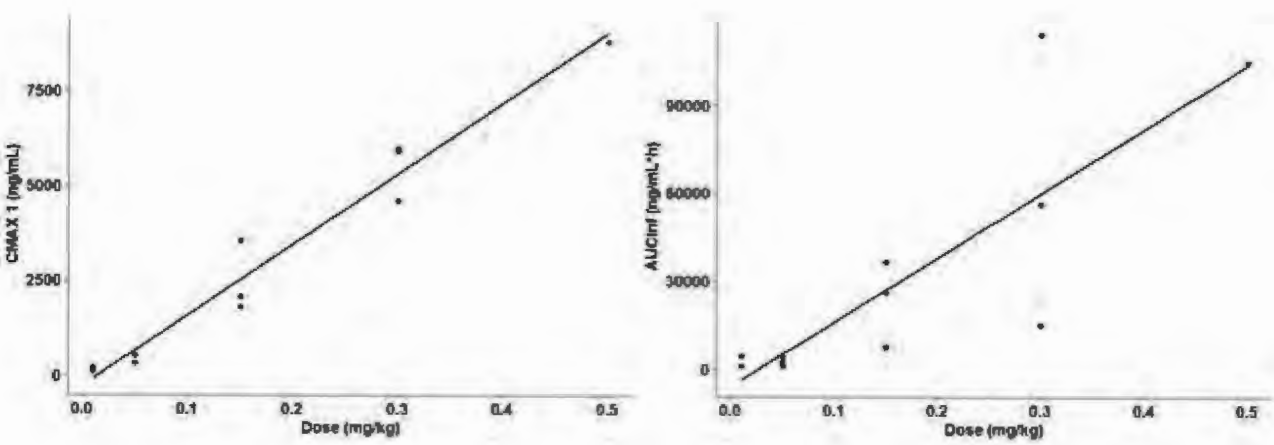
C) PK parameters of PEG_{2000}-C-DMG:

PK Parameter	0.01 mg/kg N=3	0.05 mg/kg N=3	0.15 mg/kg N=3	0.3 mg/kg N=3	0.5 mg/kg N=1
C_{max}, µg/mL	0.105 (48.5)	0.356 (21.4)	2.06 (14.2)	4.22 (9.00)	10.3 (NC)
$AUC_{0\text{-last}}$, µg•h/mL	2.32 (24.2)	12.3 (12.7)	51.0 (18.0)	87.6 (16.6)	175 (NC)
$AUC_{0\text{-}\infty}$, µg•h/mL	2.83 (27.6)	13.3 (13.0)	52.1 (18.2)	89.6 (16.8)	179 (NC)
t_{max}[a], h	1.00 (1.00-1.50)	1.00 (1.00-1.08)	1.08 (1.08-1.17)	1.00 (1.00-1.08)	2.38 (2.38-2.38)
$t_{1/2\beta}$, d	1.25 (36.4)	1.98 (10.1)	6.17 (44.7)	7.58 (31.2)	7.21 (NC)
V_{ss}[b], L/kg	0.126 (45.2)	0.191 (19.6)	0.179 (22.2)	0.213 (14.0)	0.171 (NC)
CL[b], mL/h/kg	3.15 (25.9)	3.23 (13.2)	2.50 (18.1)	2.90 (16.7)	2.38 (NC)

Values indicate mean (%CV). NC: Not calculated. Source: Module 5.3.3.1 ALNY-PCS-102, Table 13.2, Table 17.2.1.1, Table 17.2.2.1, Table 13.9, Table 13.11, Table 17.2.1.1

Dose Proportionality:

Figure 6. The relationship between dose and systemic exposures of patisiran siRNA in healthy volunteers.



Source: Module 5.3.3.5, ALNY-CSC-122PK, alnpkdataset

Patisiran siRNA showed a linear and approximately dose-proportional increase in C_{max} and AUC over the dose range of 0.01 to 0.5 mg/kg (Figure 6). Both DLin- MC3-DMA and PEG_{2000}-C-DMG also showed an approximately dose proportional increase in systemic exposures in healthy subjects.

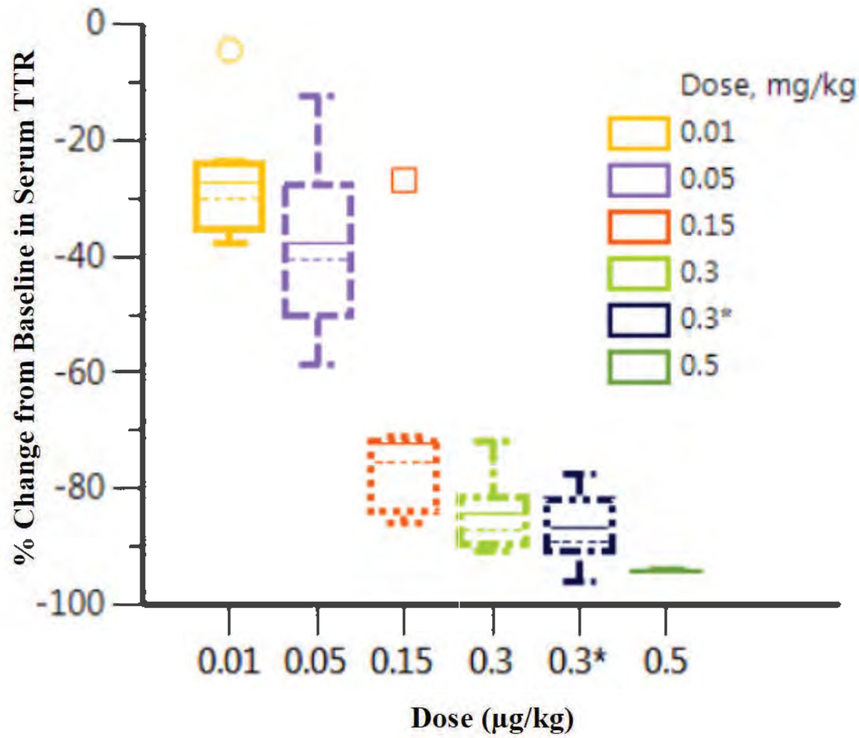
Pharmacokinetic characteristics of patisiran siRNA, DLin- MC3-DMA and PEG_{2000}-C-DMG in 12 Japanese healthy volunteers in study ALN-TTR02-005 was relatively similar to that of study ALN-TTR02-001. In the multiple dose studies (ALN-TTR02-002 and ALN-TTR02-003) in 27 patients with hATTR, patisiran exposures reached steady state by week 24 following

administration of 0.3 mg/kg Q3W. The accumulation ratio for C_{max}, AUC_{last} and C_{trough} of patisiran siRNA at steady state was 1.7, 3.2 and 3.2-fold, respectively.

Pharmadynamics:

The relationship between patisiran-LNP dose and maximum reduction in serum TTR from baseline was evaluated using data pooled from study ALN-TTR02-001, ALN-TTR02-002 and ALN-TTR02-005. Patisiran showed a dose-dependent reduction of serum TTR with a maximum reduction in serum TTR was observed at 0.3 mg/kg (Figure 7). Premedications such as hydrocortisone and antihistamine did not affect the TTR lowering effects of patisiran. Patisiran also exerted a dose-dependent reduction in Vitamin A and retinol binding protein (RBP) levels in parallel with TTR reduction. Therefore, Vitamin A supplementation is recommended for patients who receive patisiran therapy in order to alleviate any potential deficits in Vitamin A.

Figure 7. The relationship between dose and maximum reduction in serum TTR from baseline in healthy subjects and in patients with hATTR (pooled analysis of data from studies ALN-TTR02-001, ALN-TTR02-002 and ALN-TTR02-005)



*indicates patients received reduced premedication such as hydrocortisone and antihistamine. The upper and lower end of the box represents the upper and lower quartiles, and the median and mean are marked by the dotted line and solid line inside the box. The top and lower lines are the range without outliers, and the symbols are outliers. Source: Module 2.7.2. Summary of Clinical Pharmacology Studies; ALNY-PCS-102, Figure 13.10; ALNY-PCS-110, Figure 13.10; ALNY-PCS-103, Figure 10.16.

4.3 Population PK and/or PD Analyses

A meta-analysis of population pharmacokinetics of patisiran siRNA was conducted using PK data from 199 subjects including healthy subjects (n=22) from Phase 1 studies (ALN-TTR02-001 and ALN-TTR02-005), and patients with hATTR-PN (N=177) from Phase 2 studies (ALN-TTR02-002 and ALN-TTR02-003) and a Phase 3 study (ALN-TTR02-004). A brief summary of the five studies is shown below (Table 4).

Table 4. Summary of study designs used in Phase 1 studies in healthy subjects and Phase 2 and its extension studies and a Phase 3 study in patients with hATTR-PN

Study Number	Study Description	Dose regimens	Numbers of Subjects	PK Sampling
ALN-TTR02--001	A Phase 1, Randomized Single-blind, Placebo-controlled, Single-ascending Dose, Safety, Tolerability and Pharmacokinetics Study of ALN-TTR02 in Healthy Volunteers	Single IV patisiran-LNP infusion of 0.01, 0.05, 0.15, 0.3 and 0.5 mg/kg administered over 60 minutes	A total of 13 healthy volunteers received patisiran-LNP (0.01, 0.05, 0.15, 0.3 and 0.5 mg/kg). A total of 4 healthy volunteers received placebo (one in each of dose groups 0.01, 0.05, 0.15, and 0.3 mg/kg).	Pre-dose, EOI, and at 5, 10, and 30 minutes, and at 1, 2, 4, 6, 8, 24, 48 hours post infusion, and on Days 3, 4, 7, 14, 21, 28, 42, 90, and 180 post infusion as well as the early termination visit (if applicable).
ALN-TTR02--005	A Phase 1, Randomized, Double-blind, Placebo-controlled, Single-ascending Dose, Safety, Tolerability and Pharmacokinetics Study of ALN-TTR02 in Japanese Healthy Volunteers	Single IV patisiran-LNP infusion of 0.05, 0.15, 0.3 mg/kg administered over 70 minutes	A total of 9 healthy Japanese volunteers received patisiran-LNP (0.05, 0.15, and 0.3 mg/kg).	Pre-dose, EOI, and at 5, 10, and 30 minutes, and at 1, 2, 4, 6, 8, 24, 48 hours post infusion, and on Days 3, 4, 7, 14, 21, 28, 42 and 90 post infusion
ALN-TTR02--002	A Phase 2, Open-Label, Multi-Dose, Dose Escalation Trial to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Intravenous Infusions of ALN-TTR02 in Patients with TTR Amyloidosis	IV patisiran-LNP infusion of 2 consecutive doses, 4 weeks apart of the following dose levels: 0.01, 0.05, 0.15, and 0.30 mg/kg over 60 minutes. IV patisiran-LNP infusion of 2 consecutive doses at 0.30 mg/kg 3 weeks apart.	A total of 29 patients received patisiran-LNP: • 0.01 mg/kg q4w (n=4) • 0.05 mg/kg q4w (n=3) • 0.15 mg/kg q4w (n=3) • 0.30 mg/kg q4w (n=7) • 0.30 mg/kg q3w (n=12)	Pre-dose, EOI and at 5, 10 and 30 minutes and at 1, 2, 4, 6, 24 and 48 hours post-infusion on Day 0 (1st infusion) and Day 21 (q3w, 2nd infusion)/ 28 (q4w, 2nd infusion). Samples were also collected on Days 7 (35 for 2nd infusion), 14 (42), 21 (49) and Additional samples were collected on Days 84 and 180 for the q4w regimen. Additional samples were collected on Days 35, 91 and 187 for the Q3W regimen..
ALN-TTR02--003	A Phase 2, Multicenter, Open-label, Extension Study to Evaluate the Long-term Safety, Clinical Activity, and Pharmacokinetics of ALN-TTR02 in Patients with Familial Amyloidotic Polyneuropathy Who Have Previously Received ALN-TTR02	IV patisiran-LNP infusion of 0.3 mg/kg over 70 minutes once every 3 weeks during approximately 2 years and 4 months	A total of 27 patients received patisiran-LNP. Overall, 25 patients were enrolled in the PK/PD subgroup	Pre-dose, EOI, and at 1 hour post infusion, for the first dose and every dose up through, and including, the 3 month dosing visit, and then every 6, 9, 12 month dosing days. EOI on15, 18, 21, and 24 month dosing days. Patients in the PK/PD subgroup, additional samples collected at the first dosing (Day 0) and then the 8, and the 24-month dosing visits at 2, 4, and 6 hours post-infusion.
ALN-TTR02--004	APOLLO: A Phase 3 Multicenter, Multinational, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of Patisiran (ALN-TTR02) in Transthyretin (TTR)-Mediated Polyneuropathy (Familial Amyloidotic Polyneuropathy-FAP)	IV patisiran-LNP infusion of 0.3 mg/kg once every 21 (±3) days administered over 70 minutes.	A total of 148 patients received patisiran-LNP. A total of 77 patients received placebo.	Day 0, Day 126 (Week 18), Day 399 (Week 57): pre-dose and at EOI Day 21 (Week 3) and Day 252 (Week 36), Day 546 (Week 78): Predose and 30 minutes after the EOI Additional PK samples collected at any time of early withdrawal visit. For patients with rapid disease progression who discontinued study drug, PK samples were taken at any time on Day 294 and Day 546

EOI: End of infusion; Source: Population PK Reports; Module 5.3.3.5, ALNY-CSC-122PKPD

The objectives of this analysis were, 1) to develop population PK models for patisiran siRNA in healthy subjects and patients with hATTR-PN, 2) to identify covariates that explain variability in PK and quantify intra- and inter-subject variability of patisiran siRNA.

Applicant's Analysis:

Population PK Analysis:

The pooled analysis dataset for patisiran siRNA include 449 plasma concentrations from healthy volunteers and 4228 plasma concentrations from patients with hATTR amyloidosis.

Data Exclusions: A total of 44 samples with ALN-18328 concentrations from Studies 002, 003 and 004 were removed from the analysis. Plasma concentrations of patisiran siRNA below the limit of quantification (BLQ) [Table 5], subject reported extravasation, suspected sample switch, unexpectedly low/high concentration relative to similar timepoints and missing information regarding the collection time were excluded from the analysis. Given that the dataset contains >10% of BLQ on patisiran-LNP treatments, M3 likelihood estimation method was employed to account for BLQ samples.

Table 5. The number of BLQ samples in population PK dataset

Analyte	Samples Status	Healthy Subjects	Patients with hATTR Amyloidosis with Polyneuropathy	Overall
Patisiran siRNA	Total number of samples	449 (100%)	4228 (100%)	4677 (100%)
	BLQ Pre-treatment	22 (4.9%)	170 (4.0%)	192 (4.1%)
	BLQ On-Treatment	117 (26.1%)	**539 (12.7%)**	656 (14.0%)
	Non-BLQ Pre-treatment	0 (0.0%)	3 (0.1%)	3 (0.1%)
	Non-BLQ On-Treatment	310 (69.0%)	3516 (83.2%)	3826 (81.8%)

Source: Population PK Reports; Module 5.3.3.5, ALNY-CSC-122PK

Modeling Strategy: Population PK modeling and model validation were performed using Phoenix^{TM} NLME® v7 (Pharsight – A CertaraTM Company). Modeling strategy includes, 1) development of structural PK model 2) development of random effects model including between-subject (BSV) variability and residual unexplained variability, 3) evaluation of covariates that explain BSV, 4) development of final model, 5) evaluation of model adequacy [goodness-of-fit (fitted and observed concentrations, conditional weighted residuals versus time] and 6) validation of final model using bootstrapping and visual predictive check methods.

The relationship between plasma concentration and time was described in the model using,

$$C_{ij} = C\left(D_i, t_{ij}, \theta_i\right) \cdot \left(1 + \varepsilon_{p,ij}\right) + \varepsilon_{a,ij}$$
$$\theta_i = \left(\theta_{i1}, \dots, \theta_{ip}\right)$$

where C_{ij} is the concentration at the jth collection time tij for subject i, D_i represents dosing history for subject i, θ is the vector of p PK parameters for subject i, and $\varepsilon_{p,ij}$ and $\varepsilon_{a,ij}$ are the proportional and additive random residual error terms, respectively, associated with jth concentration for subject i. ε_p and ε_a are normally distributed with mean 0 and variances σp2 and σa2, respectively.

BSV was modeled using,

$$\theta_{in} = \left(\theta_{TV,n} e^{(\eta_{in})}\right)$$
$$\left(\eta_{1,\dots,}\eta_p\right) = MVN(0, \Omega)$$

where θ_{in} is the value of the nth PK parameter of the ith individual, $\theta_{TV,n}$ is the typical value of the nth PK parameter in the population, ηin is the random inter-individual deviation from the typical value θ_{TV},n for subject i. Inter-individual random effects (η1,…, ηp), also known as ETAs, are multivariate normally (MVN) distributed with mean 0 and estimated variance ωn2 included in the variance-covariance OMEGA (Ω) matrix.

Residual unexplained variability was modeled using proportional or additive and proportional models on linear or log-transformed concentration data.

Covariate analysis: Baseline characteristics of continuous covariates (6A), categorical covariates (6B) and time varying covariates (6C) in the PK population are shown in Table 6.

Table 6A. Baseline characteristics of continuous covariates in the PK population

Characteristics	Population		Total (n=199)
	Healthy Subjects (n=22)	Patients with hATTR Amyloidosis with Polyneuropathy (n=177)	
Age (years) Mean (SD) Median [Min, Max]	29.8 (6.99) 29.0 [21, 44]	59.1 (12.6) 62.0 [24, 83]	55.8 (15.2) 60.0 [21, 83]
Weight (kg) Mean (SD) Median [Min, Max]	69.7 (12.6) 72.0 [45.6, 88.4]	67.8 (16.4) 66.0 [36.2, 110]	68.0 (16.0) 68.0 [36.2, 110]
eGFR* (mL/min/1.73m^2) Mean (SD) Median [Min, Max]	115 (17.5) 114 [85.4, 152]	110 (44.2) 101 [31.1, 346]	110 (42.1) 106 [31.1, 346]
TTR (µg/mL) Mean (SD) Median [Min, Max]	278 (44.0) 281 [195, 365]	204 (67.1) 208 [52.3, 411]	213 (68.9) 215 [52.3, 411]
mNIS+7 (score) Mean (SD) Median [Min, Max] Missing	NA NA 22 (100.0%)	76.6 (41.8) 72.0 [2.00, 165] 2 (1.1%)	76.6 (41.8) 72.0 [2.00, 165] 24 (12.1%)

Table 6B. Baseline characteristics of categorical covariates in the PK population

Characteristics	Count (%)		
	Healthy Subjects (n=22)	Patients with hATTR Amyloidosis with Polyneuropathy (n=177)	Total (n=199)
Sex			
Male	21 (95.5%)	129 (72.9%)	150 (75.4%)
Female	1 (4.5%)	48 (27.1%)	49 (24.6%)
Age Group			
Adults <65 years	22 (100.0%)	102 (57.6%)	124 (62.3%)
Elderly ≥65 years	0 (0.0%)	75 (42.4%)	75 (37.7%)
Race			
Caucasian	11 (50.0%)	142 (80.2%)	153 (76.9%)
Non-Caucasian	11 (50.0%)	35 (19.8%)	46 (23.1%)
Population			
Healthy	22 (100.0%)	0 (0.0%)	22 (11.1%)
hATTR with Polyneuropathy	0 (0.0%)	177 (100.0%)	177 (88.9%)
Hepatic Function			
Normal	22 (100.0%)	161 (91.0%)	183 (92.0%)
Mild	0 (0.0%)	15 (8.5%)	15 (7.5%)
Moderate	0 (0.0%)	1 (0.6%)	1 (0.5%)
Renal Function			
Normal	20 (90.9%)	121 (68.4%)	141 (70.9%)
Mild	2 (9.1%)	38 (21.5%)	40 (20.1%)
Moderate	0 (0.0%)	18 (10.2%)	18 (9.0%)

Table 6C. Time varying covariates in the PK population

Characteristics	Healthy Subjects (n=22)	Patients with hATTR Amyloidosis with Polyneuropathy (n=177)	Overall (n=199)
Antidrug Antibodies			
Absence (Negative)	20 (90.9%)	169 (95.5%)	189 (95.0%)
Presence (Positive)[2]	2 (9.1%)	8 (4.5%)	10 (5.0%)
CYP3A Inducers			
Absence	22 (100.0%)	175 (98.9%)	197 (99.0%)
Presence[1]	0 (0.0%)	2 (1.1%)	2 (1.0%)
CYP3A Inhibitors			
Absence	22 (100.0%)	150 (84.7%)	172 (86.4%)
Presence[1]	0 (0.0%)	27 (15.3%)	27 (13.6%)
Batch Scale[3]			
7.5 kg	22 (100.0%)	29 (16.4%)	51 (25.6%)
10 kg	0 (0.0%)	175 (98.9%)	175 (87.9%)
54 kg	0 (0.0%)	41 (23.2%)	41 (20.6%)

[1] Subjects who received a CYP3A inducers or inhibitors within 14 days of a PK sample. [2] Subjects with at least one positive ADA measurement in the study. [3] Some patients received more than one batch for at least one dose and were thus included in more than one category.

Source: Population PK Reports; Module 5.3.3.5, ALNY-CSC-122PK

The covariates evaluated in this analysis are shown in Table 7.

Table 7. List of covariates that were included in covariate analysis

Covariate	Type	Purpose	Parameters[c] and Inclusion Criteria for Evaluation (F, P, or S)[b]
Patisiran-LNP batch scale (7.5-kg and 54-kg batch scale relative to 10 kg batch scale)	Time-varying categorical	Impact of batch change on relative bioavailability	Frel (S)
ADA status	Time-varying categorical	Impact of ADA on PK	CL (S)
Concomitant use of moderate or potent CYP3A inhibitors or inducers	Time-varying categorical	Impact of CYP3A on PK	CL (S)
Age	Continuous	Demographic effect on PK	CL (F), V1 (P)
Weight	Continuous	Demographic effect on PK	CL (F), V1 (F)
eGFR (up to moderate renal impairment)	Continuous	Renal impairment on PK	CL (F)
Baseline mNIS+7	Continuous	Disease status on PK	CL (P), V1 (P)
Baseline TTR	Continuous	Disease status on PK	CL (P), V1 (P)
Sex	Categorical	Demographic effect on PK	CL (F), V1 (P)
Race (Caucasian vs Non-Caucasian [a])	Categorical	Race effect on PK	CL (F), V1 (P)
Population (healthy vs patients)	Categorical	Population on PK	CL (F), V1 (P)
NCI-ODWG classification up to mild hepatic impairment	Categorical	Liver impairment on PK	CL (F)

[a] Non-Caucasian includes Asian, Black; American Indian or Alaska Native; Native Hawaiian or other Pacific Islander and others; [b] F = Formally included in the population PK model; P = Potentially included in the population PK model if significant trend is observed on the random effect plots; S = Separately evaluated in a univariate model and included in the full model if difference of the MOF significantly decrease ($p<0.01$); [c] Key PK parameters for ALN-18328 are CL12, CL20 and V1. Source: Population PK Reports; Module 5.3.3.5, ALNY-CSC-122PK

Covariates were either separately (S) evaluated in a univariate model, formally (F) included in the population PK model, or potentially (P) included in the population PK model if a significant trend was observed on the random effect plots. The time-varying covariates (ADA, moderate or strong CYP3A inhibitors and inducers, and batch scale) were included in the full model evaluation, if found statistically significant ($p<0.01$) in a univariate setting (are labeled as “S”).

Power functions were used for the inclusion of continuous covariates in the model.

$$\theta_i = \theta_{\text{Typical}} \cdot \left(\frac{Cov_i}{Cov_{reference\ value}} \right)^{\theta_{\text{eff}}}$$

where θ_i is the population value for subjects with covariate equal to Cov_i, $\theta_{Typical}$ is the typical value of the PK parameter for subjects having the covariate equal to the reference value ($Cov_{reference}$) and θ_{eff} is the effect values of the covariate on parameter θ.

Using an exponential function, categorical covariates with numerical values from 1 to n were evaluated in the model.

$$\theta i = \theta_{\text{Typical}} \cdot \exp\left(\theta_{\text{effi}} \cdot [Cov = i]\right)$$

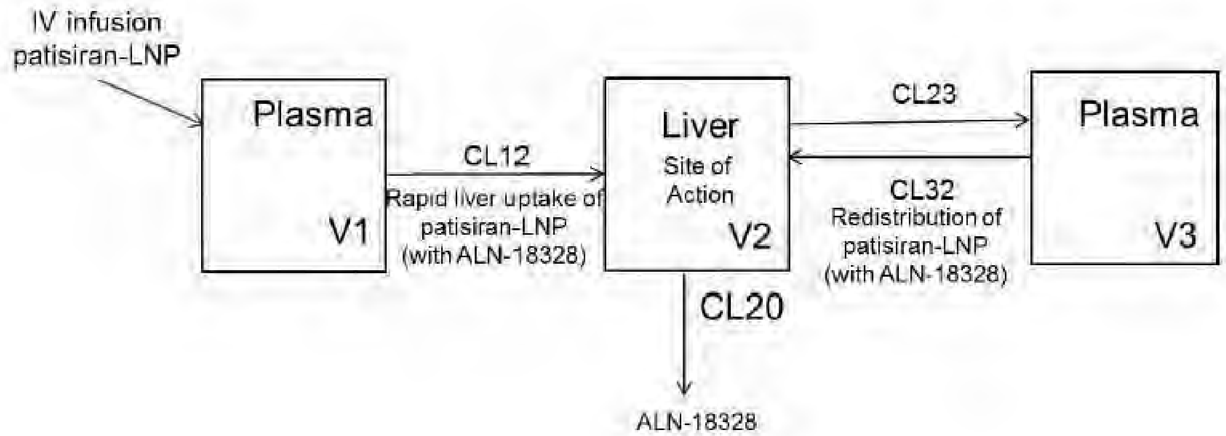
where $\theta_{Typical}$ is the population value of PK parameters for subjects in the reference category and $\exp(\theta_{effi})$ is the multiplicative effect of the category i on parameter θ.

A full covariate model was considered superior to a stepwise approach (forward inclusion and backward elimination) because it accounts for potential collinear effect of covariates. To obtain non-parametric 95% confidence intervals of the parameter estimates, 500 stratified bootstrap simulations were performed based on a full covariate model with selected covariates. The stratification factors for bootstrap included population, hepatic impairment (up to mild impairment), batch scale, sex and race to account for potential imbalance in patient characteristics. A covariate effect was statistically significant if the bootstrap derived non-parametric 95% CI of the estimate did not contain the null value. Subsequently, simulations were performed from the bootstrap derived PK parameters estimates and forest plots were generated to present the univariate effects (mean and 90% CI) of the baseline covariates on fold-changes in steady state AUC_{last},ss and C_{max},ss. The bias of each parameter was calculated by the percentage change between median value derived from the bootstrap and the full model PK estimate. No model reduction step was performed and all covariates were retained in the final population PK model.

Results:

A semi-mechanistic model for patisiran siRNA was developed to describe the pharmacokinetics of patisiran siRNA Figure 8. This model consists of plasma and liver compartments, distribution and redistribution clearances describing the active liver uptake and redistribution between plasma and liver compartments, and elimination clearance from liver compartment. The accumulation of patisiran siRNA following repeat dosing is based on accumulation of LNP which in turn depends on the individual specific accumulation of DLin-MC3-DMA.

Figure 8. Semi-mechanistic model for patisiran siRNA



CL20 = LNP (ALN-18328) elimination clearance from liver (compartment 2); CL12 = LNP (ALN-18328) initial distribution clearance from plasma (compartment 1) to liver (compartment 2) in first phase; CL23 = LNP (ALN-18328) redistribution clearance from liver (compartment 2) to plasma (compartment 3) in second phase; CL32 = LNP (ALN-18328) distribution clearance from plasma (compartment 3) to liver (compartment 2) in second phase; IV = intravenous; LNP = lipid nanoparticle; V1 = distribution volume of compartment 1; V2 = distribution volume of compartment 2; V3 = distribution volume of compartment 3. Source: Population PK Reports; Module 5.3.3.5, ALNY-CSC-122PK

The differential equations used in the model are given below:

$$\frac{d(A1)}{dt} = input - \left(\frac{CL12}{F1} \cdot C1\right)$$

$$\frac{d(A2)}{dt} = \left(\frac{CL12}{F1} \cdot C1\right) - \left(\frac{CL20}{F2} \cdot C2\right) - \left(\frac{CL23}{F2} \cdot C2\right) + \left(\frac{CL32}{F2} \cdot C3\right)$$

$$\frac{d(A3)}{dt} = \left(\frac{CL23}{F2} \cdot C2\right) - \left(\frac{CL32}{F2} \cdot C3\right)$$

$$F1 = 1 + R1 \cdot \left(1 - exp^{(-gamma * (t - TimeStart))}\right)$$

$$F2 = 1 + R2 \cdot \left(1 - exp^{(-gamma * (t - TimeStart))}\right)$$

Where F1 and F2 are siRNA accumulations in first and second phases and are governed by the accumulation of DLin-MC3-DMA. The gamma term represents the rate of terminal elimination phase of DLin-MC3-DMA.

$$C1 = \frac{A1}{V1/F1}$$

$$C2 = \frac{A2}{V2/F2}$$

$$C3 = \frac{A3}{V1/F2}$$

$$C13 = C1 + C3$$

where A1 = amount in compartment 1 (plasma compartment); A2 = amount in compartment 2 (liver compartment); A3 = amount in compartment 3 (plasma compartment); C1 = concentration in compartment 1; C2 = concentration in compartment 2 (hepatic compartment); C3 = concentration in compartment 3; CL12 = Clearance from compartment 1 to compartment 2; CL20 = hepatic clearance; CL23 = clearance from compartment 2 to compartment 3; CL32 = clearance from compartment 3 to compartment; V1 = distribution volume of compartment 1; V2 = distribution volume of compartment 2; V3 = distribution volume of compartment 3; F1 = relative bioavailability in compartment 1, F2 = relative bioavailability in compartment 2; R1 = accumulation ratio parameter for the first phase; R2 = accumulation ratio parameter for the second phase; t = time; TimeStart = time since start of treatment; Gamma = rate of accumulation of DLin-MC3-DMA; C13 is the total sum of concentrations (C1 and C3) in the plasma compartments.

The parameter estimates of typical CL12 and CL20 of patisiran siRNA were 1.82 and 0.0752 L/h, respectively. BSV of CL12 and CL20 were approximately 36% and 83%, respectively. Following incorporation of all covariates, the BSV of CL12 and CL20 was decreased by 6.6% and 5.4%, respectively. The residual variability was 52% and it was estimated using proportional error model. The accumulation ratio for the first (1+R1) and second phases (1+R2) were 1.2 and 3.2, respectively (Table 8).

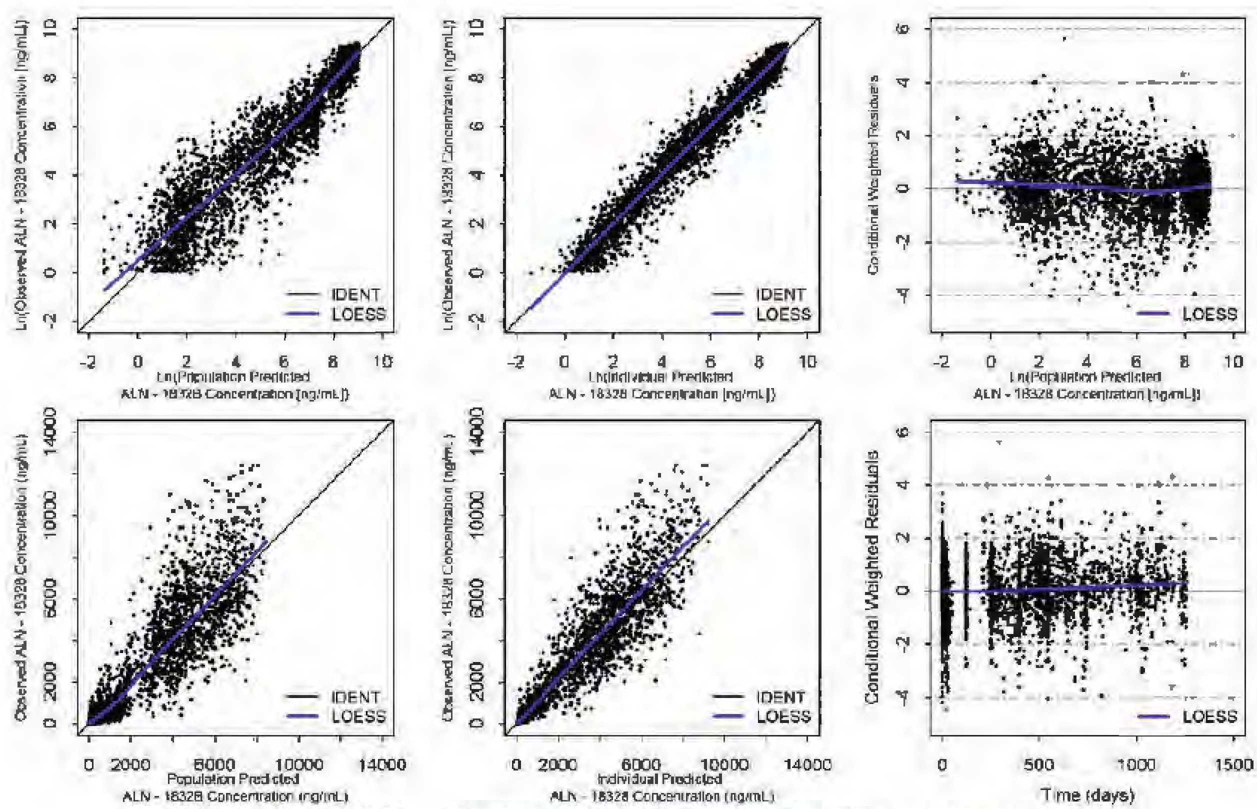
The parameter estimates from full PK model for patisiran siRNA and the statistical significance of the parameter estimates are shown in Table 8.

Table 8. Parameter estimates of patisiran siRNA from final PK model

PK Parameters	Population Estimates	BSV (%)	Shrinkage (%)	Lower* 95% CI	Upper* 95% CI	Statistical Significance[a]
CL12 (L/h)	**1.82**	**36.0**	**20.6**	**1.69**	**1.97**	**NA**
Weight	$\times(\text{Weight}/66)^{0.774}$			0.551	1.07	SS
Healthy	×1.56			1.16	2.03	SS
CL20 (L/h)	**0.0752**	**82.7**	**13.9**	**0.0592**	**0.0964**	**NA**
Age	$\times(\text{Age}/62)^{-0.00976}$			-0.0203	0.00233	NS
Weight	$\times(\text{Weight}/66)^{-0.175}$			-0.978	0.728	NS
eGFR**	$\times(\text{eGFR}/101)^{-0.0106}$			-0.0266	0.000407	NS
Mild HI***	× 1.09			0.706	1.78	NS
Sex (if female)	× 1.01			0.996	1.02	NS
Race (if non-Caucasian)	× 0.988			0.972	0.997	SS
Healthy	× 0.899			0.805	1.01	NS
CL23 (L/h)	**0.00278; fixed**	**NA**	**NA**	**NA**	**NA**	**NA**
CL32 (L/h)	**0.0249; fixed**	**NA**	**NA**	**NA**	**NA**	**NA**
R1	0.224	**NA**	**NA**	0.158	0.313	**NA**
R2	2.19	**NA**	**NA**	1.6	2.92	**NA**
V1 (L)	2.27	16.5	39.7	2.15	2.42	**NA**
Weight	$\times(\text{Weight}/66)^{0.258}$			0.0949	0.486	SS
V2 (L)	**1; fixed**	**NA**	**NA**	**NA**	**NA**	**NA**
Frel						
Batch scale 10 kg	1; fixed	NA	NA	NA	NA	NA
Batch scale 7.5 kg	×0.733			0.684	0.796	SS
Batch scale 54 kg	×0.867			0.775	1.03	NS
Error Model						
Log Additive Error	0.521			0.483	0.56	

BSV = between-subjects variability; CL12 = clearance from compartment 1 to compartment 2; CL20 =hepatic clearance; CL23 = clearance from compartment 2 to compartment 3; CL32 = clearance from compartment 3 to compartment 2; eGFR = estimated glomerular filtration rate (mL/min/1.73 m2); Frel = bioavailability relative to batch scale 10 kg, hATTR = hereditary TTR-mediated amyloidosis; HI = hepatic impairment; F1= (1+R1) = accumulation ratio for the first phase; F2 = (1+R2) = accumulation ratio for the second phase; V1 = distribution volume of compartment 1; V2 = distribution volume of compartment 2; V3 = distribution volume of compartment 3; NA = not applicable; NS = not statistically significant; SS = statistically significant; * Non-parametric 95% confidence intervals derived based on bootstrap resampling; ** eGFR values were capped to 150 mL/min1.73 m2; *** One subject with moderate hepatic impairment was pooled with subjects with mild hepatic impairment; Statistical significance reached when nonparametric 95% CIs excluded the null value; PK parameters presented for a typical patient defined as follows: A male of 62 years of age Caucasian patient with hATTR amyloidosis with polyneuropathy, with a body weight of 66 kg, eGFR of 101 mL/min/1.73m2, and with normal hepatic function. Source: Population PK Reports; Module 5.3.3.5, ALNY-CSC-122PK

Figure 9. Goodness-of-fit of the full PK model for patisiran siRNA



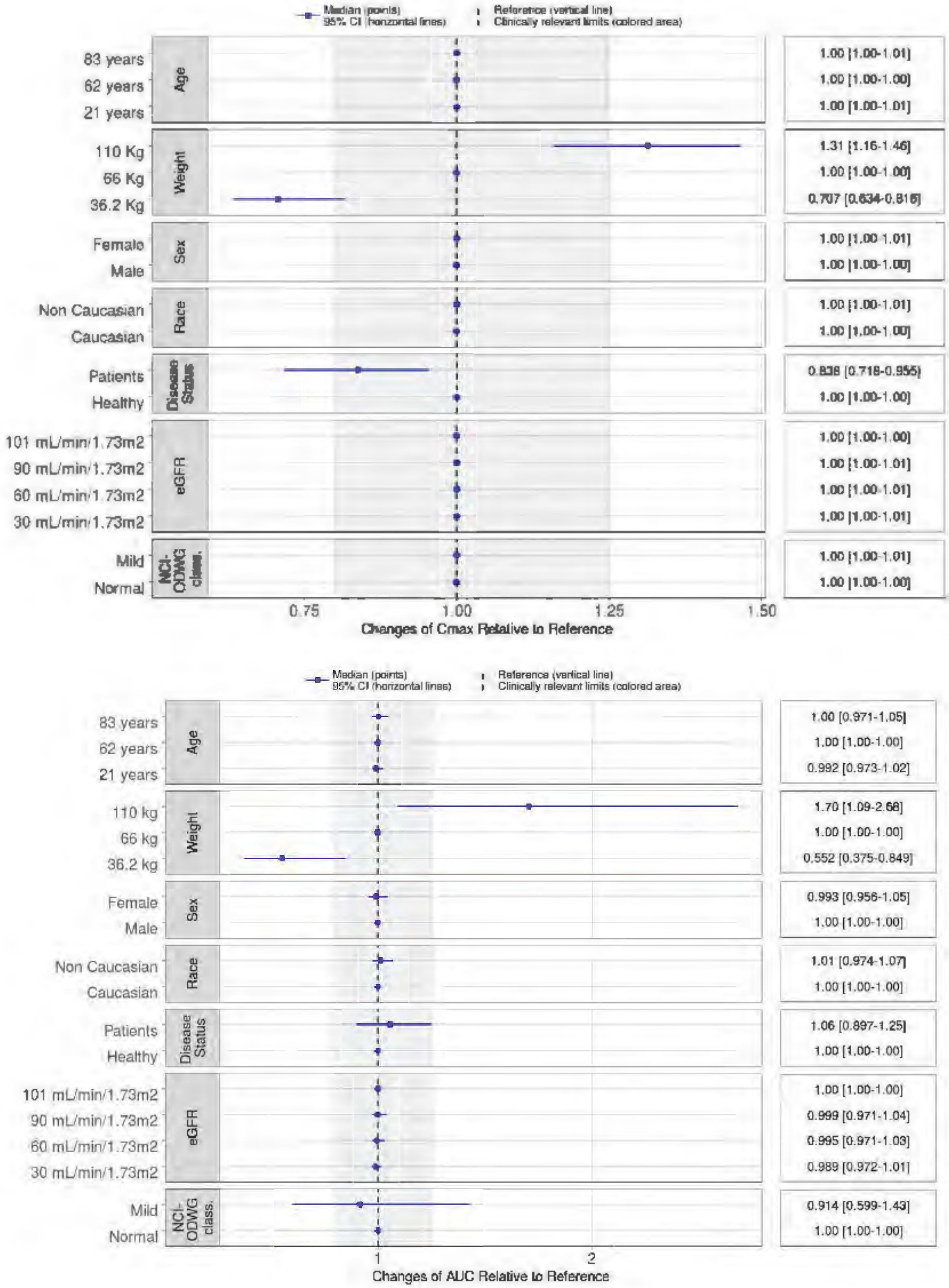
IDENT = identity line; LOESS = locally weighted scatterplot smoothing; Ln = natural log-transformed

Source: Population PK Reports; Module 5.3.3.5, ALNY-CSC-122PK

Both population predicted and individual predicted concentrations of patisiran siRNA were well fitted with the full PK model (Figure 9). This suggests that model can reasonably predict the plasma concentration-time of patisiran siRNA in patients with hATTR-PN. The model predicted PK parameters for patisiran siRNA were shown in (Table 9).

Covariates include baseline age, sex, race (Caucasian versus non-Caucasian), renal function (mild and moderate impairment), mild hepatic function impairment, 54-kg batch scale, ADA, and concomitant administration of moderate or strong CYP3A inhibitors/inducers did not affect the PK of patisiran siRNA. Systemic exposures ($AUC_{last,\ ss}$) to patisiran siRNA were increased with increasing body weight. The predicted $AUC_{last,\ ss}$ in a patient with a body weight of 36.2 kg is approximately 45% lower than a patient with body weight of 66 kg. The predicted $AUC_{last,\ ss}$ in a patient with a body weight of 110 kg is approximately 70% higher than a patient with body weight of 66 kg (Figure 10).

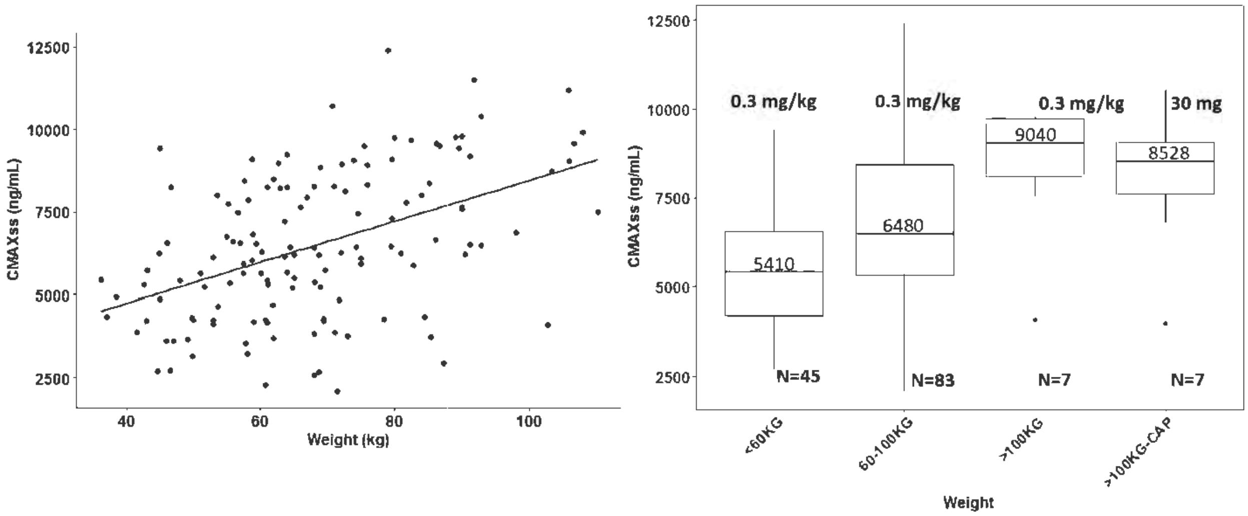
Figure 10. Forest plot of covariates effect on steady state C_{max} and AUC_{last} of patisiran siRNA



The dots and the horizontal segments represent bootstrap-derived mean and 95% CI of covariate effect relative to the reference patient (i.e., a male of 62 years of age Caucasian patient with hATTR amyloidosis with polyneuropathy, with a body weight of 66 kg, eGFR of 101 mL/min/1.73m2, and with normal hepatic function). The shaded area represents effect size of 80% -125%. Source: Population PK Reports; Module 5.3.3.5, ALNY-CSC-122PK

There was a trend towards increasing exposures ($C_{max,ss}$) to patisiran siRNA with increasing body weight following administration of 0.3 mg/kg Q3W in patients with hATTR-PN. In patients weighing >100 kg, the exposures ($C_{max,ss}$) observed following administration of 0.3 mg/kg dosing regimen were comparable to exposures normalized to 30 mg equivalent dose (Figure 11).

Figure 11. Effect of body weight on patisiran siRNA exposures



>100KG-CAP indicates $CMAX_{ss}$ values were normazlied to 30 mg equivalent dose of patisiran-LNP in patients who weigh >100 kg; Source: Study ALN-TTR02-004; Module 5.3.5.1

The impact of missing BLQ values on PK parameter and covariate estimation using final PK model was assessed using a sensitivity analysis. The typical values of CL12, CL20, R1, R2, and V1 derived with the M3 method were all within 10% of those derived in the original analysis. Covariate effects derived with the M3 method were all within 12% of those derived in the original analysis. This suggests that missing BLQ values did not have a significant effect on PK parameter or covariate estimation.

Table 9. Model predicted AUC_{last}, C_{max}, C_{avg} and C_{trough} following first dose and steady state for patients with hATTR-PN in study ALN-TTR02-004

PK Day	Parameters	Mean	SD	Geo Mean	%Geo CV	5th Perc.	25th Perc.	Median	75th Perc.	95th Perc.
First Dose	AUC_{tau1} (μg*h/mL)	52.3	30.2	45.9	52.2	23.2	31.1	41.5	62.4	121
Steady State	$AUC_{tau,ss}$ (μg*h/mL)	130	86.1	110	60.7	51.8	70.1	104	156	337
First Dose	C_{max1} (μg/mL)	5.08	0.931	5.00	18.7	3.67	4.33	4.99	5.82	6.68
Steady state	$C_{max,ss}$ (μg/mL)	6.08	1.27	5.95	21.1	4.25	5.24	5.85	6.86	8.58
First Dose	C_{ave1} (μg/mL)	0.104	0.0599	0.0911	52.2	0.0460	0.0618	0.0824	0.124	0.240
Steady State	$C_{ave,ss}$ (μg/mL)	0.258	0.171	0.218	60.7	0.103	0.139	0.206	0.309	0.669
First Dose	$C_{trough1}$ (μg/mL)	0.00674	0.0108	0.00341	153	0.000703	0.00154	0.00287	0.00699	0.0274
Steady State	$C_{trough,ss}$ (μg/mL)	0.0110	0.0174	0.00508	213	0.000976	0.00229	0.00474	0.0110	0.0485

Source: Population PK Reports; Module 5.3.3.5, ALNY-CSC-122PK

Reviewer comments: The final model predicted concentrations match well with the observed plasma concentrations of patisiran siRNA. The conditional weighted residuals (CWRES) vs population predicted concentration plot shows that CWRES are evenly distributed around 0. These results suggest that the final PK model adequately describes the PK of patisiran siRNA. The model-predicted PK parameter estimates are relatively similar to that obtained using non-compartmental analysis. No covariates but body weight were identified to influence the PK of patisiran siRNA. Because patisiran is dosed based on body weight, and the exposures observed following administration of 0.3 mg/kg dosing are comparable to the exposures normalized to 30 mg equivalent dosing, the dose of patisiran is capped to 30 mg in patients weighing over 100 kg. Overall, the proposed 0.3 mg/kg Q3W dosing for patients weighing up to 100 kg and 30 mg Q3W dosing for patients weighing over 100 kg are acceptable. Further, the dose adjustment of patisiran-LNP is not necessary based on age, gender, race, renal function (mild and moderate impairment), mild hepatic function impairment, ADA status, and concomitant administration of moderate or strong CYP3A inhibitors/inducers.

Population PK/PD Analysis:

A population PK/PD analysis of patisiran siRNA was conducted using PK/PD data from 283 subjects including healthy subjects (n=22 patisiran siRNA and 7 placebo) from Phase 1 studies (ALN-TTR02-001 and ALN-TTR02-005), and patients with hATTR-PN (N=177 patisiran siRNA and 77 placebo) from Phase 2 studies (ALN-TTR02-002 and ALN-TTR02-003) and a Phase 3 study (ALN-TTR02-004). A brief summary of the study design used in these studies are described in Table 4.

The objectives of this analysis were, 1) to develop PK/PD model to assess the relationship between patisiran siRNA concentrations and serum TTR lowering effects in healthy subjects and patients with hATTR-PN, 2) to identify covariates that explain variability in serum TTR lowering effects and quantify intra- and inter-subject variability of PD response.

Data: A total of 4331 measurable serum TTR concentrations from both placebo treated and patisiran-LNP treated subjects were available for PK/PD modeling. All subjects who received at least one dose of patisiran-LNP or placebo and had at least one measurable serum TTR concentration were included in PK/PD analysis.

Software: Population PK/PD modeling and model evaluation and simulations were performed using PhoenixTM NLME® v7 (Pharsight – A CertaraTM Company).

Data Exclusions: Overall, 4331 serum TTR samples were retained in the PK/PD analysis. Six samples had absolute value of CWRES higher than 4 based on the structural population PK/PD model were excluded from the analysis.

Modeling Strategy: Patisiran-LNP degrades TTR mRNA thereby inhibiting the production of both wild-type and mutant TTR protein in hepatocytes. Therefore, an indirect response PK/PD model linking patisiran siRNA plasma concentrations to reduction of synthesis rate of TTR through an effect compartment was used to describe the down-regulation of liver TTR mRNA. An effect compartment was added to a semi-mechanistic model developed for population PK analysis of patisiran siRNA. Effect compartment is an empirical equilibrium compartment between plasma patisiran siRNA concentrations and the concentrations at the effect site. An effect compartment was necessary to describe the hysteresis or delayed onset and longer duration of effect observed following patisiran-LNP infusion.

The relationship between serum TTR concentrations and plasma concentrations of patisiran siRNA was described in the model using,

$$C_{ij} = C\left(D_i, t_{ij}, \theta_i\right) \cdot \left(1 + \varepsilon_{p,ij}\right) + \varepsilon_{a,ij}$$
$$\theta_i = \left(\theta_{i1}, \ldots, \theta_{ip}\right)$$

where C_{ij} is the concentration at the jth collection time t_{ij} for subject i, D_i represents dosing history for subject i, θ is the vector of p PD parameters for subject i, and $\varepsilon_{p,ij}$ and $\varepsilon_{a,ij}$ are the proportional and additive random residual error terms, respectively, associated with jth concentration for subject i. ε_p and ε_a are normally distributed with mean 0 and variances σp2 and σa2, respectively.

BSV was modeled using equations similar to that described in the modeling strategy section of population PK analysis. The residual unexplained variability was modeled using proportional residual models.

Covariate Analysis:

Baseline characteristics of continuous covariates (10A) and categorical covariates (10B) in the PK population are shown in Table 10.

Table 10A. Baseline characteristics of continuous covariates in the PD population

Descriptive Statistics		Healthy Subjects		Patients with hATTR amyloidosis with polyneuropathy		Overall	
		Patisiran-LNP (n=22)	Placebo (n=7)	Patisiran-LNP (n=177)	Placebo (n=77)	Patisiran-LNP (n=199)	Placebo (n=84)
Serum TTR (µg/mL)	Mean (SD)	278.5 (43.97)	299.2 (41.43)	204.4 (67.11)	198.8 (58.08)	212.6 (68.93)	207.2 (63.18)
	Median [Min, Max]	280.6 [195, 365]	294.4 [241, 371]	208.5 [52.3, 411]	196.4 [58.5, 320]	215.1 [52.3, 411]	201.7 [58.5, 371]
mNIS+7 score	Mean (SD)	NA NA	NA NA	76.63 (41.80)	74.61 (37.04)	76.63 (41.80)	74.61 (37.04)
	Median [Min, Max]	NA NA	NA NA	72.00 [2.00, 165]	71.50 [11.0, 154]	72.00 [2.00, 165]	71.50 [11.0, 154]
	Missing	22 (100%)	7 (100%)	2 (1.1%)	0 (0.0%)	24 (12.1%)	7 (8.3%)
Age (years)	Mean (SD)	29.8 (7.0)	27.7 (8.9)	59.1 (12.6)	62.2 (10.8)	55.8 (15.2)	59.3 (14.3)
	Median [Min, Max]	29.0 [21, 44]	26.0 [21, 47]	62.0 [24, 83]	63.0 [34, 80]	60.0 [21, 83]	62.0 [21, 80]
Body Weight (kg)	Mean (SD)	69.69 (12.61)	79.21 (19.72)	67.83 (16.39)	67.50 (15.72)	68.04 (16.00)	68.47 (16.28)
	Median [Min, Max]	72.00 [45.6, 88.4]	78.80 [53.8, 110.2]	66.00 [36.2, 110.3]	67.40 [40.8, 99.0]	68.00 [36.2, 110.3]	68.00 [40.8, 110.2]
eGFR (mL/min/ 1.73 m²)	Mean (SD)	115 (17.2)	107 (19.1)	110 (44.2)	104 (34.3)	110 (42.1)	104 (33.2)
	Median [Min, Max]	114 [85.4, 152]	101 [86.1, 147]	101 [31.1, 346]	97.5 [31.6, 228]	106 [31.1, 346]	98.5 [31.6, 228]

Table 10B. Baseline characteristics of categorical covariates in the PD population

Descriptive Statistics		Healthy Subjects		Patients with hATTR amyloidosis with polyneuropathy		Overall	
		Patisiran-LNP (n=22)	Placebo (n=7)	Patisiran-LNP (n=177)	Placebo (n=77)	Patisiran-LNP (n=199)	Placebo (n=84)
Sex	Male	21 (95.5%)	7 (100%)	129 (72.9%)	58 (75.3%)	150 (75.4%)	65 (77.4%)
	Female	1 (4.5%)	0 (0.0%)	48 (27.1%)	19 (24.7%)	49 (24.6%)	19 (22.6%)
Race	Caucasian	11 (50.0%)	3 (42.9%)	142 (80.2%)	50 (64.9%)	153 (76.9%)	53 (63.1%)
	Non-Caucasian	11 (50.0%)	4 (57.1%)	35 (19.8%)	27 (35.1%)	46 (23.1%)	31 (36.9%)
Hepatic Function	Normal	22 (100%)	6 (85.7%)	161 (91.0%)	75 (97.4%)	183 (92.0%)	81 (96.4%)
	Mild HI	0 (0.0%)	1 (14.3%)	15 (8.5%)	2 (2.6%)	15 (7.5%)	3 (3.6%)
	Moderate HI	0 (0.0%)	0 (0.0%)	1 (0.6%)	0 (0.0%)	1 (0.5%)	0 (0.0%)
Renal Function	Normal	20 (90.9%)	6 (85.7%)	122 (68.9%)	49 (63.6%)	142 (71.4%)	55 (65.5%)
	Mild	2 (9.1%)	1 (14.3%)	37 (20.9%)	23 (29.9%)	39 (19.6%)	24 (28.6%)
	Moderate	0 (0.0%)	0 (0.0%)	18 (10.2%)	5 (6.5%)	18 (9.0%)	5 (6.0%)
TTR Genotype	None	22 (100%)	7 (100%)	0 (0.0%)	0 (0.0%)	22 (11.1%)	7 (8.3%)
	V30M	0 (0.0%)	0 (0.0%)	78 (44.1%)	40 (51.9%)	78 (39.2%)	40 (47.6%)
	Non V30M	0 (0.0%)	0 (0.0%)	99 (55.9%)	37 (48.1%)	99 (49.7%)	37 (44.0%)
TTR Stabilizer on Treatment	Yes	0 (0.0%)	0 (0.0%)	18 (10.2%)	1 (1.3%)	18 (9.0%)	1 (1.2%)
	No	22 (100%)	7 (100%)	159 (89.8%)	76 (98.7%)	181 (91.0%)	83 (98.8%)
ADA*	Absence	20 (90.9%)	7 (100.0%)	169 (95.5%)	75 (97.4%)	189 (95.0%)	82 (97.6%)
	Presence	2 (9.1%)		8 (4.5%)	2 (2.6%)	10 (5.0%)	2 (2.4%)

ADA= anti-drug antibodies; hATTR= hereditary amyloid transthyretin; HI = hepatic impairment; LNP= lipid nanoparticles; N = number of subjects; TTR = transthyretin; V30M= valine to methionine mutation at position 30 in human TTR gene. Note: Baseline values at Study 003 were used for the descriptive statistics when available; if not values from Study 002 were used. Subjects with at least one sample with positive ADA during the trial were considered as positive. *Subjects with at least one sample with positive ADA during the trial were considered as positive. Source: Population PK/PD Reports; Module 5.3.3.5, ALNY-CSC-122PKPD

Because of the PK of patisiran siRNA was not influenced by renal function or ADA, the effects of ADA and renal function on the PD parameters of TTR were not evaluated. The list of covariates evaluated in the covariate analysis of PK/PD model is shown in Table 11.

Table 11. List of covariates that were included in covariate analysis

Covariate Effect	Type	Purpose	PD Parameter and Inclusion in the PK Model (F or P)[b]
Baseline serum TTR	Continuous	Impact on production and % change from baseline	IC_{50} (F)
Baseline mNIS+7	Continuous	Impact on production and % change from baseline	IC_{50} (P)
V30M genotype	Categorical	Impact on production and % change from baseline	IC_{50} (P)
Age	Continuous	Demographic (elderly) effect on production of TTR and % change from baseline	IC_{50} (P)
Sex	Categorical	Gender effect on production of TTR and % change from baseline	IC_{50} (P)
Race (Caucasian vs Non-Caucasian[a])	Categorical	Race effect on production of TTR and % change from baseline	IC_{50} (P)
Study population (healthy subjects vs patients with hATTR amyloidosis with polyneuropathy)	Categorical	Disease status on production of TTR and % change from baseline	IC_{50} (P)
Mild hepatic impairment (based on NCI-ODWG classification)	Categorical	Hepatic function on production of TTR and % change from baseline	IC_{50} (F)
Concomitant use of tetramer stabilizers vs no concomitant use of tetramer stabilizers	Categorical	Impact on elimination of TTR	K_{out} (P)
Patisiran-LNP batch scale (7.5 kg vs 10 kg, 10 kg vs 54 kg batch scale)	Time-varying categorical	Impact of batch change on drug effect	IC_{50} (S)

hATTR= hereditary amyloid transthyretin; Kin = zero-order rate of formation/synthesis of serum TTR; IC50 = ALN-18328 concentration producing 50% of maximal inhibition; Kout = first-order rate of degradation of serum TTR; mNIS+7= modified neuropathy impairment score +7; NCI-ODWG = National Cancer Institute Organ Dysfunction Working Group criteria; PK = pharmacokinetic; TTR = transthyretin; V30M= valine to methionine mutation at position 30 in human TTR gene. [a] Non-Caucasian will include the races of Asian, Black; American Indian or Alaska Native; Native Hawaiian or other Pacific Islander and others, and unknown (if any). [b] F = Formally included in the population PK/PD model; P = Potentially included in the population PK/PD model if significant trend is observed on the random effect plots; S= Separately evaluated in a univariate model and if 95% CI of the typical effect excluded the null value. Source: Population PK/PD Reports; Module 5.3.3.5, ALNY-CSC-122PKPD

Continuous covariates were included in the model using power functions and categorical covariates with numerical values from 1 to n were tested in the model using an exponent function as described before in covariate analysis section of the population PK analysis. The full model approach was used and all covariates entered the model simultaneously (i.e., multivariate approach) and no model reduction step was performed.

Full model parameter estimates and 95% percentile intervals (PIs) reflecting the confidence intervals (CI) were obtained via stratified non-parametric bootstrapping. The following stratification was considered to account for potential imbalance in patient characteristics: study

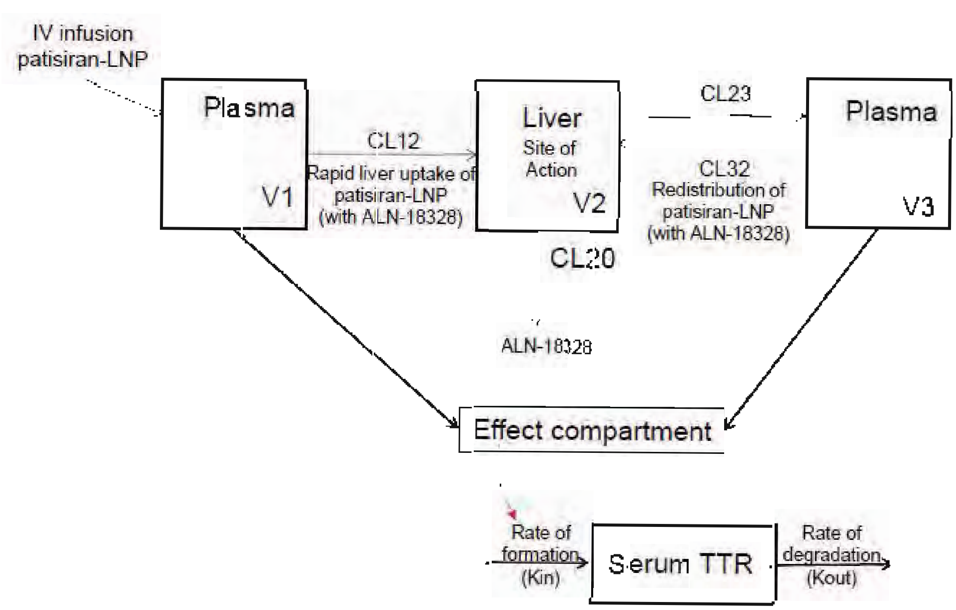
population (healthy subjects vs. patients with hATTR amyloidosis with polyneuropathy), sex, race (Caucasian/non-Caucasian), hepatic function (mild hepatic impairment vs normal), presence or absence of V30M genotype. A covariate effect was considered statistically significant if the non-parametric 95% CI of the estimate did not contain the null value. The bias of each parameter estimate was calculated by the percentage change between median value derived from the bootstrap and the full model PK/PD estimate. A sensitivity analysis was performed to evaluate the impact of missing data on the final model parameter estimates. Forest plots were used to present the univariate effects of estimated covariates and associated 90% CI on PK parameters. Fold-changes in PD parameters were computed for covariate categories of interest relative to reference patient (a 62 year-old Caucasian patient with hATTR amyloidosis with polyneuropathy weighing 66 kg with normal hepatic function, non V30M genotype and baseline serum TTR of 208 µg/mL). For continuous covariates, the minimum and maximum were used.

Dose-response simulations for patisiran-LNP were performed for the reference patient at 0.01, 0.05, 0.15, 0.3 and 0.5 mg/kg administered over 70 minutes as an IV infusion q3w. The simulated serum TTR levels at 0.3 mg/kg q3w were compared to the serum TTR levels observed in studies 003 and 004. To support the maximum dose of 30 mg in patients weighing greater than 100 kg, serum TTR levels were simulated following 30 mg q3w and compared between for typical patients weighting 100 and 120 kg.

Results:

An indirect response PK/PD model with a sigmoidal maximal inhibition (I_{max}) function inhibiting the rate of TTR formation (K_{in}) was developed to quantify the concentration-effect relationship between patisiran siRNA and serum TTR (Figure 12). This model included an effect compartment with BSV term on IC_{50}, K_{in} and K_{out} and with I_{max} fixed to 1.

Figure 12. Schematic representation of indirect response PK/PD model



CL20= elimination clearance from compartment 2; CL12= clearance from compartment 1 to compartment 2; CL23= clearance from compartment 2 to compartment 3; CL32= clearance from compartment 3 to compartment 2; Kin = rate of TTR formation; Kout = rate of TTR degradation; IV= intravenous; LNP= lipid nanoparticles; TTR = transthyretin; V1= distribution volume of compartment 1; V2= distribution volume of compartment 2; V3= distribution volume of compartment 3. Source: Population PK/PD Reports; Module 5.3.3.5, ALNY-CSC-122PKPD

Differential equation used for the population PK/PD model of serum TTR is shown below.

$$\frac{\mathrm{dTTR}}{\mathrm{dt}} = (\mathrm{K_{in}} \times DrugEffect_{\mathrm{ALN-18328}}) - \mathrm{K_{out}} \times \mathrm{TTR}$$

Where K_{in} is the zero-order formation rate of serum TTR, K_{out} is the first-order degradation rate of serum TTR. DrugEffectALN-18328 describes the concentration-effect relationship of patisiran siRNA concentrations in the effect compartment (Ce) or in the plasma compartment (compartments 1 and 3) on K_{in}. Drug effect was explored using maximum inhibition effect model (I_{max}) as presented below.

$$DrugEffect_{\mathrm{ALN-18328}} = \left(1 - I_{max} \bullet \frac{Ce(t)^{\gamma}}{{IC_{50}}^{\gamma} + Ce(t)^{\gamma}}\right)$$

Where Ce(t) is the concentration in the effect site at time "t"; IC_{50} is the concentration in the effect compartment that results in 50% lowering of the synthesis rate (or 50% lowering of serum TTR); parameter γ is the Hill-coefficient and describes the shape of the concentration-effect curve relationship.

Patisiran siRNA concentration profile in the effect compartment is described using the following differential equation:

$$\frac{dCe(t)}{dt} = K_e \times (C13(t) - Ce(t))$$

The equation above describes the equilibration between plasma patisiran siRNA concentrations in central compartment C13 (predicted from the population PK model) with the effect compartment (Ce) through equilibrium rate constant (Ke).

Baseline covariates were evaluated on PD parameters of IC_{50} and K_{in} using a full covariate model approach. The value of K_{in}, which reflects the rate of endogenous TTR production, was 2.86 µg/mL/h and the rate of TTR degradation (K_{out}) of 0.0178 h-1 (corresponding to a half-life of 40 h) was estimated for a typical patient. Baseline serum TTR, which is the ratio of Kin/Kout was 161 µg/mL. This value was slightly lower than the median baseline serum TTR concentration of 208.5 µg/mL. The estimate of Hill-coefficient of sigmoidicity was 0.548. The IC_{50} of patisiran siRNA for serum TTR reduction was 9.45 ng/mL with a BSV of 344%. The residual variability on predicted serum TTR concentrations was 28.2%. The patisiran-LNP

concentrations for 80% (IC_{80}) and 90% (IC_{90}) TTR reduction are estimated to be 118.5 and 520.5 ng/mL.

The parameter estimates from full PK/PD model for patisiran siRNA and the statistical significance of the parameter estimates are shown in Table 12.

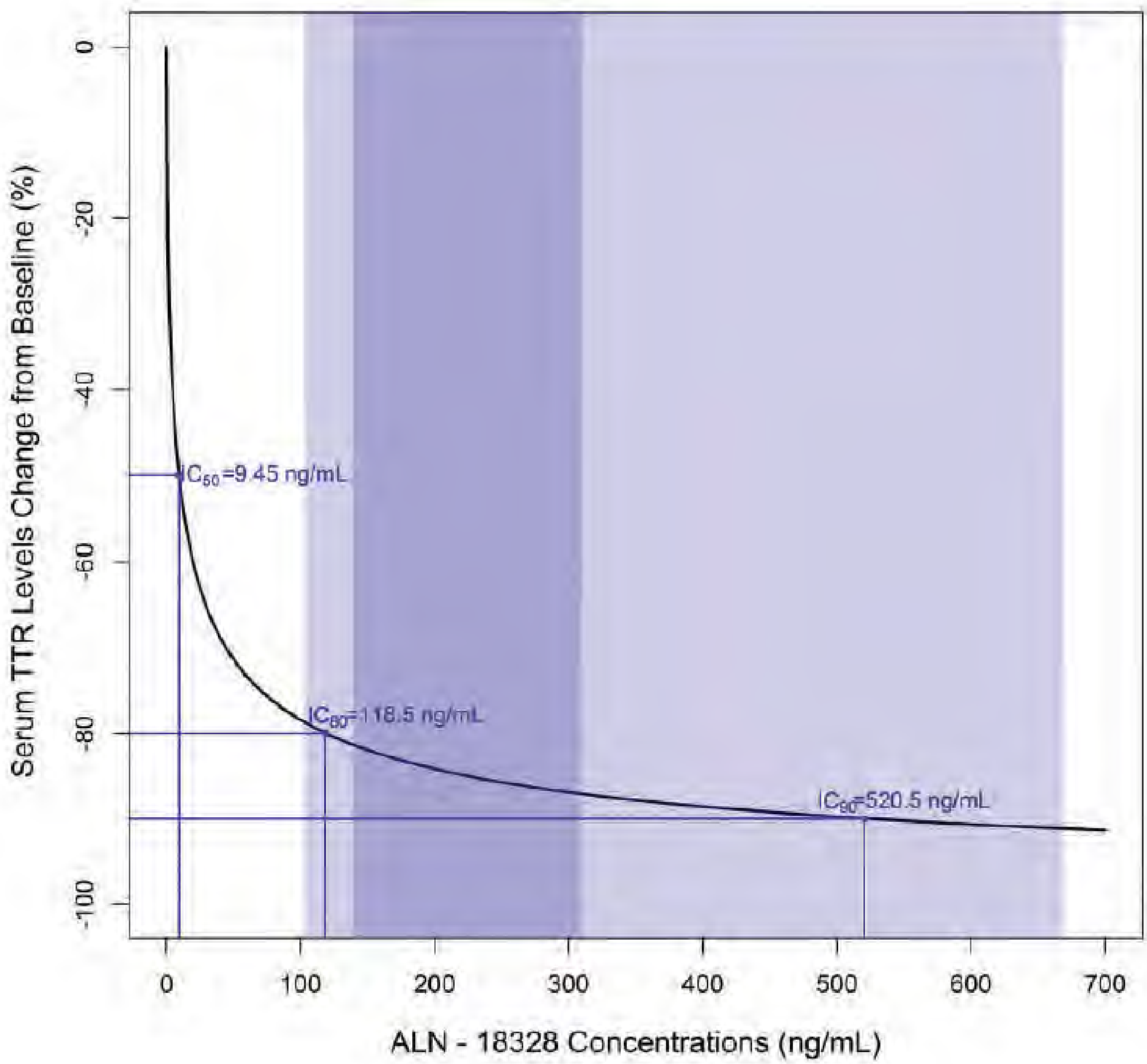
Table 12. Parameter estimates of patisiran siRNA on serum TTR from final PK/PD model

Parameters	Population Estimates	BSV (%)	Shrinkage (%)	Lower* 95% PI	Upper* 95% PI	Statistical Significance
Ke (h^{-1})	0.00551	0 FIX	NA	0.0046	0.0074	NA
K_{in} (μg/mL/h)	2.86	50.8	44.2	2.32	3.43	NA
Body weight (kg)	× $(Weight/66)^{0.370}$			0.179	0.467	SS
Age (years)	× $(Age/62)^{-0.00846}$			-0.0105	-0.0080	SS
If healthy subjects	× 1.62			1.51	1.77	SS
V30M genotype	× 1.34			1.25	1.45	SS
If Mild HI*	× 0.981			0.971	0.983	SS
K_{out} (h^{-1})	0.0178	42.2	62.9	0.0145	0.0211	NA
I_{max}	0.999 (FIX)			NA	NA	NA
IC_{50} (ng/mL)	9.45	344	21.4	3.75	14.2	NA
Baseline TTR (μg/mL)	× $(TTR/208)^{0.864}$			-0.833	3.26	NS
Body weight (kg)	× $(Weight /66)^{0.910}$			0.529	2.56	SS
Age (years)	× $(Age/62)^{-0.0197}$			-0.0255	-0.0195	SS
If Mild HI	× 0.689			0.500	0.910	SS
Hill (gamma)	0.548			0.422	0.635	NA
Error Model						
Proportional Error (%)	28.2			26.5	29.8	NA

BSV= between-subject variability; Ce= concentration of ALN-18328 in the effect compartment; hATTR= hereditary amyloid transthyretin; HI= hepatic impairment; IC50 = ALN-18328 concentration producing 50% of maximal inhibition; Imax = maximal inhibition; Ke = transfer rate constant to the effect compartment; Kin = rate of TTR formation; Kout = rate of TTR degradation; NA = not applicable; PD = pharmacodynamic; PK = pharmacokinetic; PI = percentile interval; TTR = transthyretin; SS= statistically significant; V30M= valine to methionine mutation at position 30 in human TTR gene. Note 1: Indirect Kin/Kout model was used to described time profiles of serum TTR $\frac{dTTR}{dt} = K_{in} \times \left[\frac{Imax \times Ce^{\gamma}(t)}{IC50^{\gamma} + Ce^{\gamma}(t)}\right] - K_{out} \times TTR$. Note 2: One subject with moderate hepatic impairment was pooled with subjects with mild hepatic impairment. * Statistical significance reached when nonparametric 95% PIs excluded the null value. PD parameters presented for a typical patient defined as follows: A 62 years of age Caucasian patient with hATTR amyloidosis with polyneuropathy, with a body weight of 66 kg, non-V30M mutation, with normal hepatic function and a baseline serum TTR of 208 μg/mL. Source: Population PK/PD Reports; Module 5.3.3.5, ALNY-CSC-122PKPD

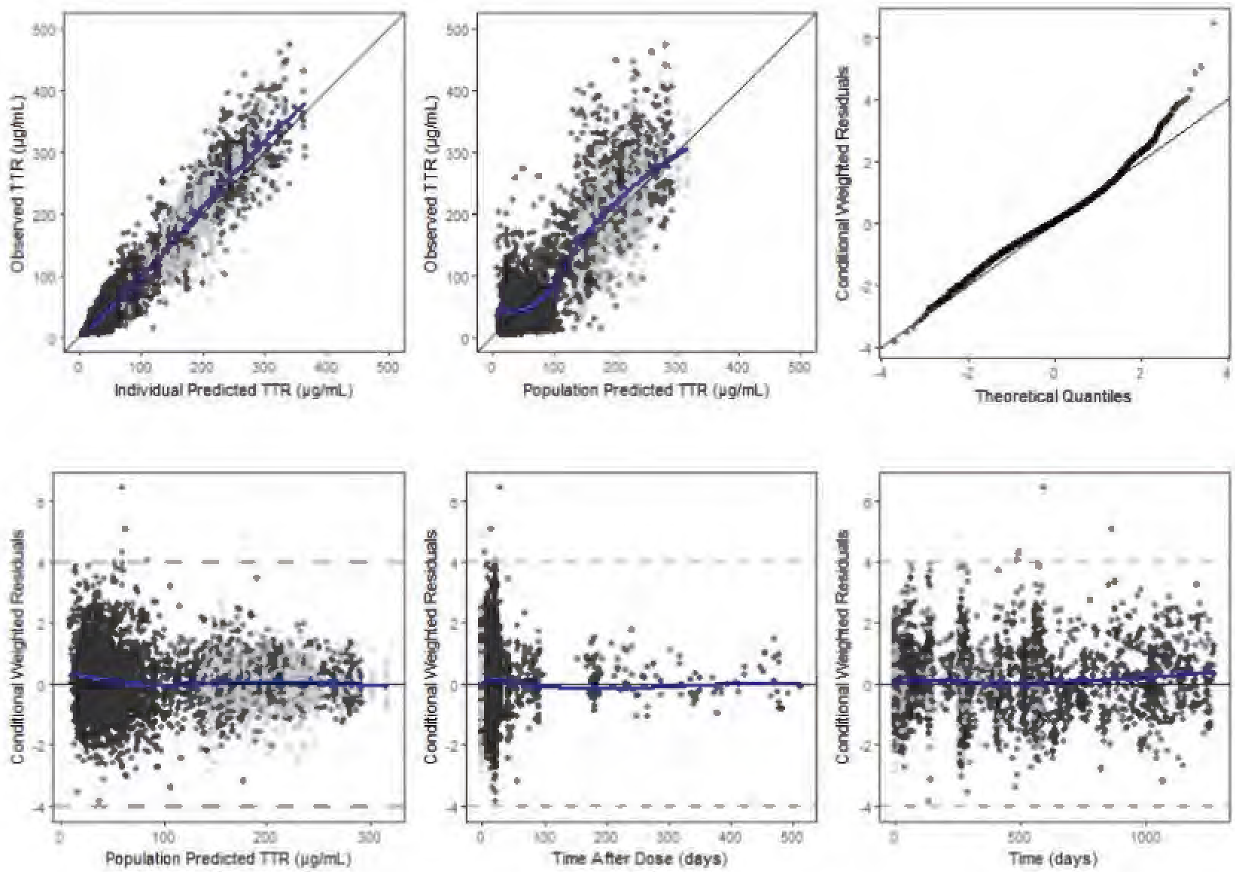
The reductions in serum TTR were positively correlated with increasing plasma concentrations of patisiran siRNAFigure 13. Steady state concentrations of patisiran siRNA (Cp_{ave} of 248 ng/mL) is in the plateau portion of the concentration-TTR response curve.

Figure 13. Relationship between steady state plasma concentrations of patisiran siRNA and serum TTR level.



IC_{50} = ALN-18328 concentration producing 50% of maximal inhibition; IC_{80}= ALN-18328 concentration producing 80% of maximal inhibition; IC_{90} = ALN-18328 concentration producing 90% of maximal inhibition; V30M= valine to methionine mutation at position 30 in human TTR gene. Note 1: Typical relationship based on a 62 years of age Caucasian patient with hATTR amyloidosis with polyneuropathy, with a body weight of 66 kg, non-V30M mutation, with normal hepatic function and a baseline serum TTR of 208 µg/mL. Note 2: The dark blue shaded area represents the 25th-75th percentiles (139.1-309.5 ng/mL) and the light blue shaded area represents the 5th- 95th percentiles of average concentration of ALN-18328 (102.8– 668.7 ng/mL). Source: Population PK/PD Reports; Module 5.3.3.5, ALNY-CSC-122PKPD

Figure 14. Goodness-of-fit of the full PK/PD model for patisiran siRNA



Blue lines represent LOESS lines, black dots represent observations in active group and gray dots represent observations in placebo group. LOESS = locally weighted scatterplot smoothing; TTR = transthyretin. Source: Population PK/PD Reports; Module 5.3.3.5, ALNY-CSC-122PKPD

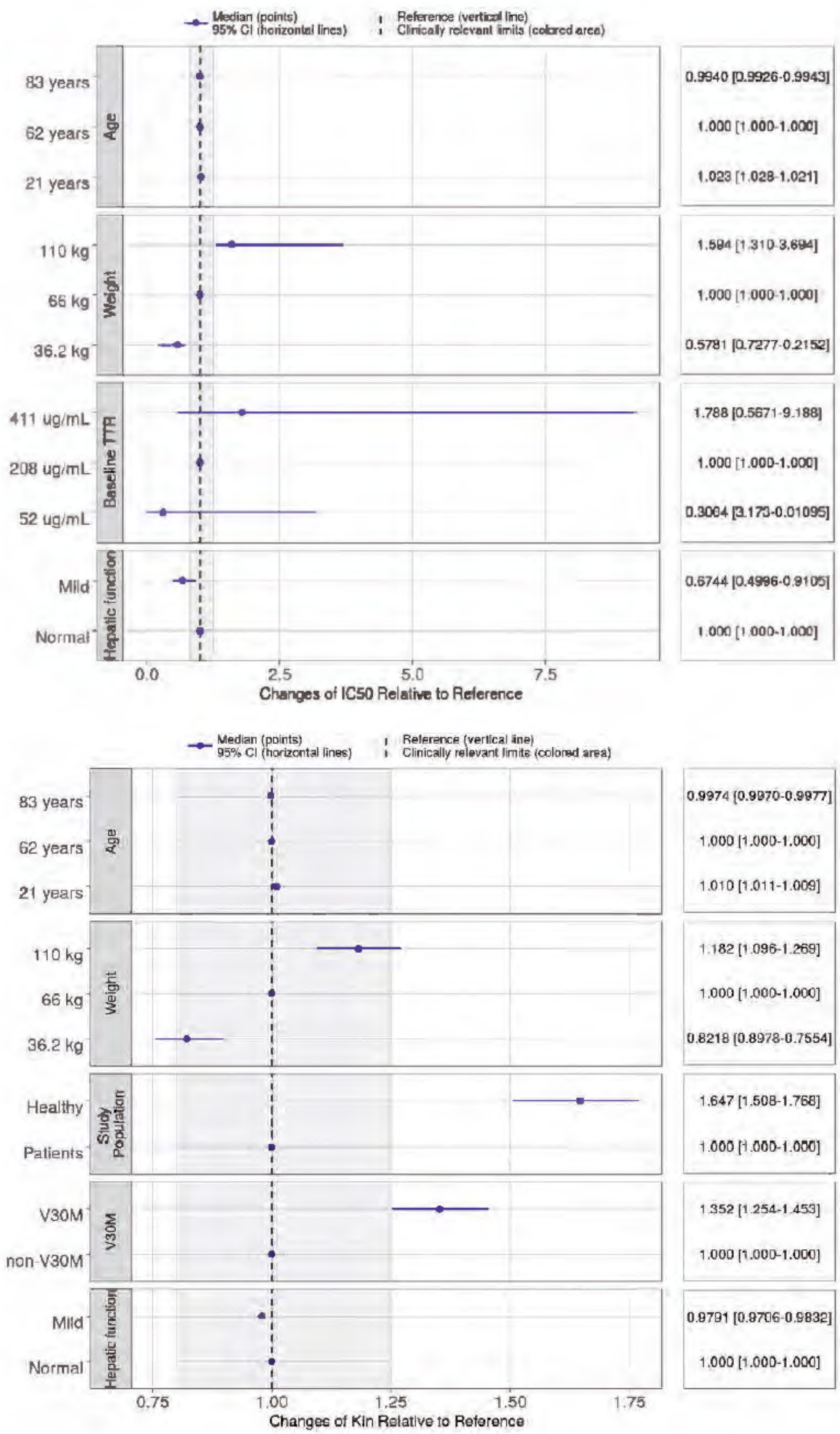
Goodness of fit plots indicate that population and individual predicted concentrations of serum TTR were well fitted with the final model. This suggests that the model can adequately predict the serum TTR lowering effects based on plasma concentrations of patisiran siRNA.

Covariates effect on K_{in}: Healthy subjects are estimated to have a 62% higher baseline serum TTR (260 µg/mL). Age had a minor effect (1%) on K_{in}. The synthesis rate of TTR increased with increasing body weight and was higher in patients with V30M genotype. The magnitude of these effects were <35% (Figure 15).

Covariates effect on IC_{50}: The covariates included in the IC_{50} (i.e., baseline serum TTR, body weight, age and hepatic function) did not influence the variability of this parameter (Figure 15).

The typical value of Ke was 0.00551 h-1, which represent an equilibrium $t_{1/2}$ of 5.24 days. No variability was added on this PD parameter. Sensitivity analysis inform that the missing values did not significantly (the relative differences were less than 2%) affect PD estimates.

Figure 15. Forest plot of covariates effect on IC_{50} and K_{in} of patisiran siRNA



hATTR= hereditary amyloid transthyretin; Kin = rate of TTR formation; TTR= transthyretin; V30M= valine to methionine mutation at position 30 in human TTR gene. Reference: A 62 years of age Caucasian patient with hATTR amyloidosis, with a body weight of 66 kg, non-V30M, with normal hepatic function and a baseline serum TTR of 208 µg/mL. Source: Population PK/PD Reports; Module 5.3.3.5, ALNY-CSC-122PKPD

PK and PD simulations were conducted to evaluate the adequacy of the maximum patisiran-LNP dose of 30 mg for the patients weighing greater than 100 kg. TTR reduction was compared between 120 kg patients receiving 30 mg Q3W and 100 kg patients receiving 0.3 mg/kg Q3W (Table 13).

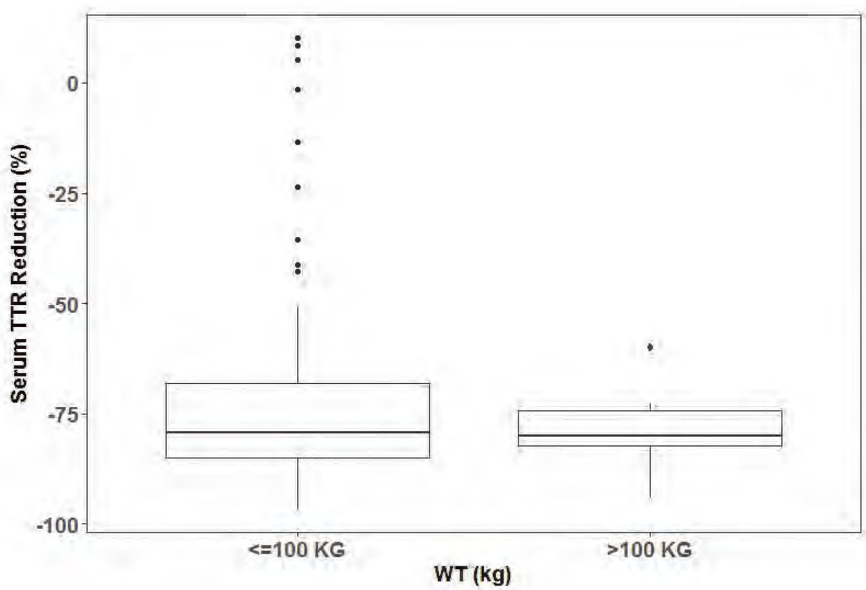
Table 13. Serum TTR Reduction in typical patients with body weight of 100 kg and 120 kg

Body weight	Parameters	5th Percentile	Median	95th Percentile
100 kg (0.3 mg/kg)	Nadir TTR (% change from baseline)	96.5	86.1	59.0
	Pre-dose TTR (% change from baseline)	94.5	78.8	45.9
120 kg (30 mg)	Nadir TTR (% change from baseline)	96.3	85.1	53.5
	Pre-dose TTR (% change from baseline)	94.4	77.6	42.2

Source: Population PK/PD Reports; Module 5.3.3.5, ALNY-CSC-122PKPD

Additionally, a model independent analysis informs that serum TTR lowering effects observed in patients weighing ≤100 kg were comparable to that observed in patients weighing >100 kg (Figure 16).

Figure 16. Effect of weight on patisiran-LNP mediated serum TTR reduction



Source: Study ALN-TTR02-004; Module 5.3.5.1

Overall, the median serum TTR lowering effects were similar between patients with body weight of ≤100 kg and patients with body weight of >100 kg. Therefore, the maximum dose of 30 mg patisiran-LNP was proposed for patients weighing >100 kg.

Reviewer comments: The final model predicted serum TTR concentrations match well with the observed concentrations of TTR. The conditional weighted residuals (CWRES) vs population predicted TTR concentration plot shows that CWRES are evenly distributed around 0. These results suggest that the final PK/PD model adequately describes the PD effects of patisiran siRNA. Given steady state plasma concentrations of patisiran siRNA are in plateau portion of concentrations-TTR response curve, almost all patients are expected to achieve approximately 80% or greater TTR lowering effects. Therefore, the interpatient variability is not expected to affect the TTR lowering effects of patisiran siRNA.

Reviewer's analysis:

Aim

- To verify population pharmacokinetic analyses evaluating the effects of different intrinsic and extrinsic factors on the PK of patisiran siRNA.
- To verify population PK/PD model assessing the relationship between patisiran siRNA concentrations and serum TTR lowering effects in healthy subjects and patients with hATTR-PN and the covariate effects on PD of patisiran siRNA
- To verify labeling statements proposed by the Applicant

Data

The datasets (alnpkdataset.xpt, dlinpkdataset.xpt, pegpkdataset.xpt, pkpddata.xpt) submitted by the Applicant were used for the analysis.

Software

Phoenix™ NLME® v7 (Pharsight – A CertaraTM Company) was used for the analysis.

Analysis Strategy

To execute the base and final population PK and PK/PD models to verify Applicant reported pharmacokinetic parameters and relationship between concentrations-serum TTR response.

Findings

The reviewer was able to run both the base and final model and derive similar PK and PD parameter estimates to those reported by the sponsor. The Applicant's proposed labeling statements about covariate effects on systemic exposures to patisiran-LNP and serum TTR lowering effects are acceptable.

4.4 Exposure-Response Analyses

Exposure-Efficacy:

As patisiran-LNP was evaluated at one dose level (0.3 mg/kg Q3W) in pivotal phase 3 trial, the adequate characterization of exposure-response analyses were limited. The percent TTR reduction was compared at different quartiles of exposures of patisiran siRNA Table 14.

Table 14. Percent reduction in TTR at different quartiles of exposures of patisiran siRNA

Parameter	Quartile 1 (N=34)	Quartile 2 (N=34)	Quartile 3 (N=34)	Quartile 4 (N=33)
C_{max_ss}, µg/mL (SD) (range)	3.69 (0.68) (2.09 to 4.66)	5.63 (0.456) (4.70 to 6.27)	7.25 (0.682) (6.28 to 8.25)	9.43 (0.945) (8.27 to 12.4)
ΔTTR, %, by C_{max_ss} (SD)	-79.4 (13.1) (-30 to -93)	-77.2 (13.3) (-37 to -94)	-76.4 (23.3) (38 to -95)	-79.4 (13.6) (-19 to -94)
$C_{p\_ss\,(30min)}$, µg/mL (SD) (range)	3.17 (0.62) (1.50 to 3.84)	4.29 (0.20) (3.85 to 4.66)	5.32 (0.46) (4.66 to 6.06)	7.15 (0.92) (6.07 to 10.3)
ΔTTR, %, by $C_{p\_ss(30min)}$ (SD) (range)	-76.1 (15.6) (-30 to -94)	-77.8 (22.5) (38 to -95)	-79.6 (9.44) (-60 to -94)	-77.1(15.7) (-19 to -95)
C_{trough_ss}, µg/mL (SD) (range)	0.0007 (0.0007) (0.000-0.002)	0.0033 (0.0009) (0.002-0.005)	0.0093 (0.0028) (0.005-0.014)	0.0838 (0.2577) (0.014-1.553)
ΔTTR, %, by C_{trough_ss} (SD) (range)	-79.4 (11.5) (- 49 to-95)	-81.4 (9.14) (-41 to- 93)	-80.2 (12.1) (-37 to -95)	-69.3 (25.3) (38 to - 91)

Source: ALN-TTR02-004 Study Reports; Module 5.3.5.1, Table 42

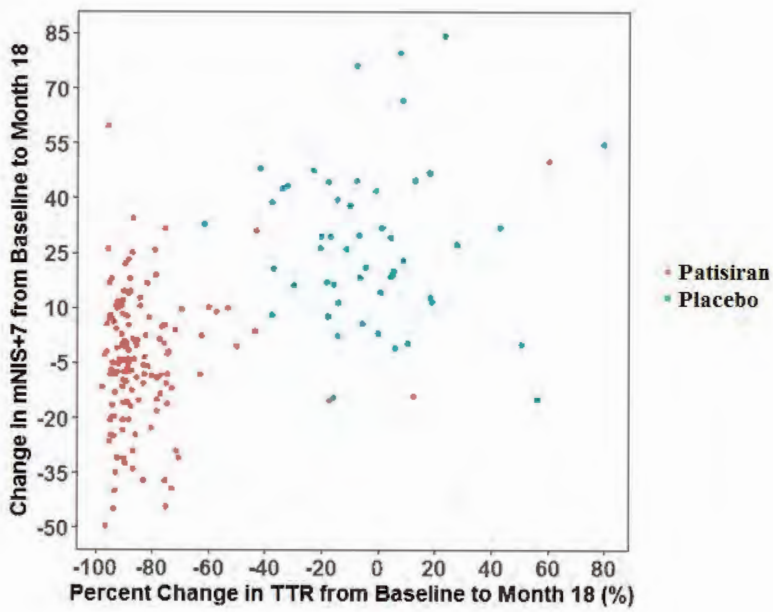
Similarly, change from baseline to Month 18 in mNIS+7 score was assessed at different quartiles of exposures of patisiran siRNA (Table 15).

Table 15. Percent change from baseline to Month 18 in mNIS+7 at different quartiles of exposures of patisiran siRNA

Parameter	Mean (SD) (Range)			
	Quartile 1 N=34	Quartile 2 N=34	Quartile 3 N=34	Quartile 4 N=33
C_{max_ss}, µg/mL	3.69 (0.678) (2.09 to 4.66)	5.63 (0.456) (4.70 to 6.27)	7.25 (0.682) (6.28 to 8.25)	9.43 (0.945) (8.27 to 12.4)
Δ mNIS+7, %, by C_{max_ss}	-2.83 (16.9) (-39.8 to 31.6)	-5.53 (20.5) (-45.0 to 59.6)	-9.24 (19.6) (-49.5 to 50.0)	1.00 (11.3) (-31.0 to 23.3)
$C_{p\_ss(30min)}$, µg/mL	3.17 (0.621) (1.50 to 3.84)	4.29 (0.196) (3.85 to 4.66)	5.32 (0.460) (4.66 to 6.06)	7.15 (0.923) (6.07 to 10.3)
Δ mNIS+7, %, by $C_{p\_ss(30min)}$	-1.64 (21.9) (-44.3 to 59.6)	-5.70 (19.3) (-49.5 to 50.0)	-6.50 (16.3) (-39.8 to 25.1)	-2.79 (14.7) (-39.4 to 31.6)
C_{trough_ss}, µg/mL	0.0007 (0.0007) (0.000 to 0.002)	0.0033 (0.0009) (0.002 to 0.005)	0.0093 (0.0028) (0.005 to 0.014)	0.0838 (0.2578) (0.014 to 1.55)
Δ mNIS+7, %, by C_{trough_ss}	-5.79 (16.0) (-45.0 to 59.6)	-0.42 (16.0) (-39.8 to 34.5)	-5.76 (16.6) (-49.5 to 31.1)	-4.94 (18.2) (-39.4 to 50.0)

Source: ALN-TTR02-004 Study Reports; Module 5.3.5.1, Table 43

Figure 17. Association between serum TTR reductions and change from baseline to Month 18 in mNIS+7



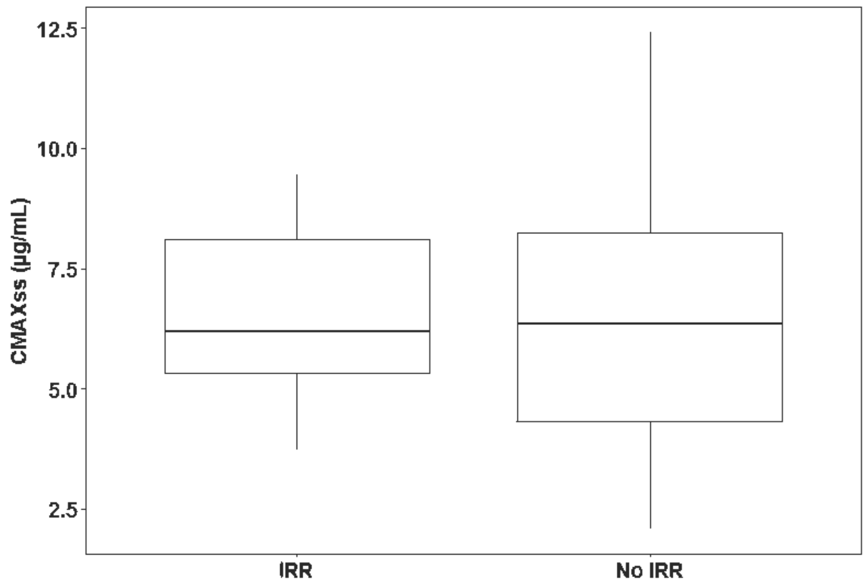
Source: Study ALN-TTR02-004; Module 5.3.5.1

Exposure-Safety:

The most frequently observed adverse reactions (≥10% of patients and occurring ≥3 percentage points higher frequency) resulting in interruption of patisiran-LNP therapy are infusion related reactions (IRR) in study ALN-TTR02-004. A total of 28 (18.9%) out of 148 patients treated

with patisiran-LNP experienced at least one IRR, while 7 (9.1%) out of 77 placebo-treated patients experienced at least 1 IRR. The first IRR events occurred within the first four dose administrations. One (0.7%) out of 148 patients discontinued the patisiran-LNP therapy due to IRR of moderate severity. The systemic exposures (C_{max}) at steady state were compared between patients who showed IRR and patients who did not show IRR (Figure 18).

Figure 18. Comparison of $C_{max,ss}$ between patients with IRR and patients without IRR



Source: Study ALN-TTR02-004; Module 5.3.5.1

Reviewer comment: *The percent reductions of TTR were similar across the exposure quartiles of patisiran siRNA which suggests that systemic exposures at different exposure quartiles were in the plateau portion of the concentration-TTR response curve in all patients. However, change from baseline to Month 18 in mNIS+7 scores ranged from 1 to -9.2 across the exposure quartiles of patisiran siRNA which indicates that factors other than TTR may also contribute for the development of neuropathy in patients with hATTR-PN. Although there is a trend towards reduction in neuropathy associated with reductions in TTR in patients with hATTR-PN, this relationship could not be adequately characterized because only one dose level was studied in pivotal phase 3 trial. The $C_{max,ss}$ of patisiran siRNA in patients with IRR was similar to that observed in patients without IRR suggest that the occurrence of IRR was independent of systemic exposures of patisiran siRNA. Overall, 0.3 mg/kg Q3W dosing regimen is adequate for all patients with hATTR-PN.*

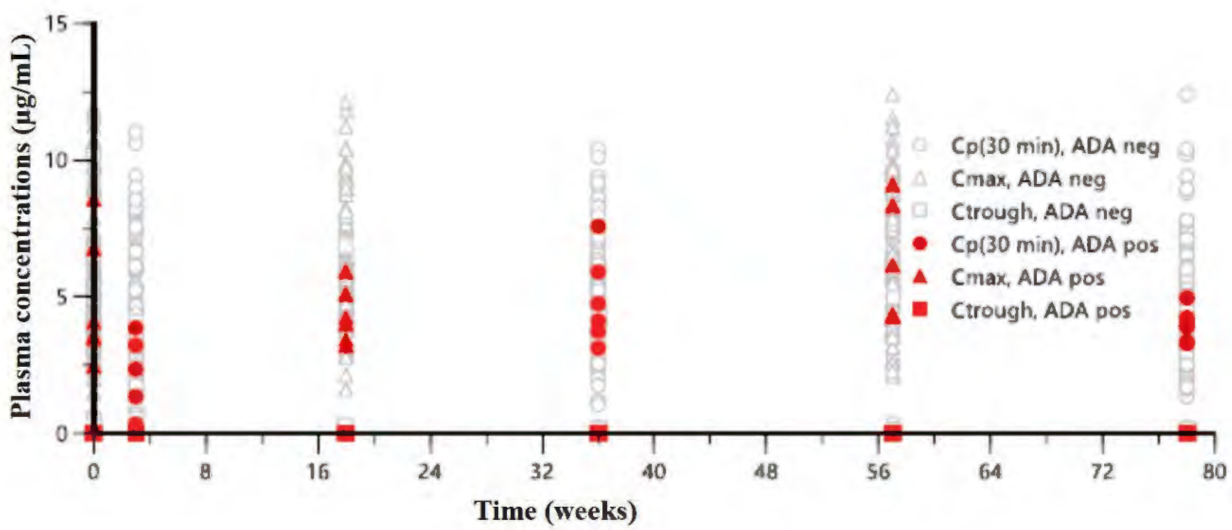
4.5 Effect of Immunogenicity on PK, PD and Efficacy

In study ALN-TTR02-004, overall 8 patients tested positive for ADAs against PEG_{2000}-C-DMG. Six patients (4.1%) were in the patisiran-LNP treatment group and 2 patients (2.6%) were in the placebo group. Two patients (1 patisiran and 1 placebo) tested positive ADAs at baseline. The

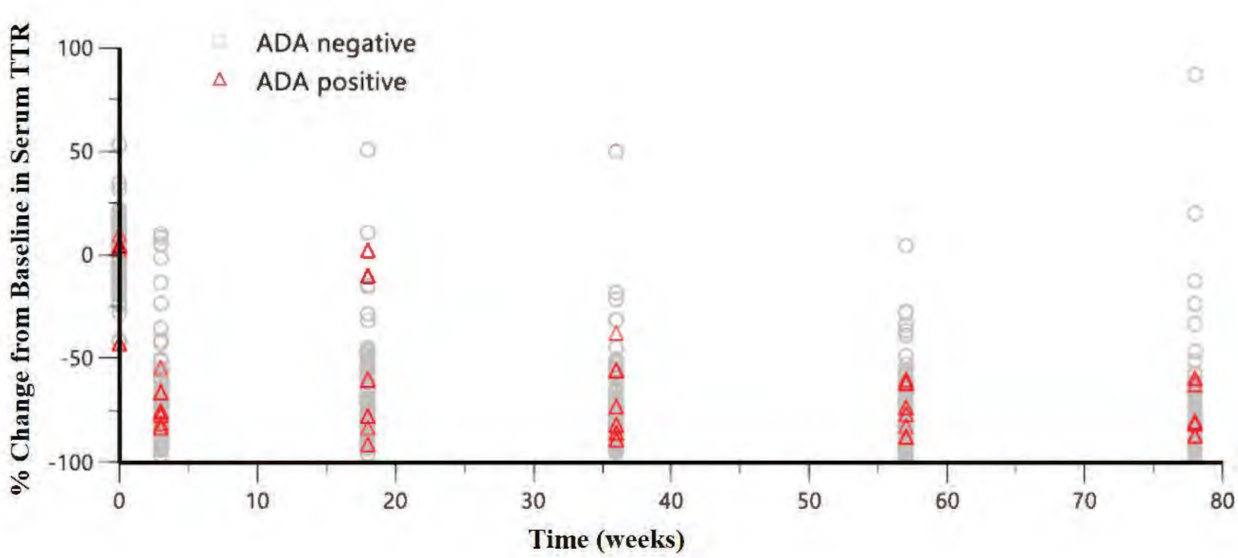
ADAs were detected in 4 patients by day 21 and in 2 patients by day 126 after the treatment with patisiran-LNP. Furthermore, the ADA titers were also low ranging from 40-80 and the appearance was transient. There was no detectable ADA after day 21 or 126 in patients on patisiran-LNP. Refer to the immunogenicity assay review by the Office of Biotechnology Products for details regarding the immunogenicity assay review.

Figure 19. Impact of immunogenicity on PK (A) and PD (B) of patisiran siRNA

A)



B)



Source: Study ALN-TTR02-004; Module 5.3.5.1

Figure 20. Impact of immunogenicity on efficacy of patisiran-LNP

	ADA Positive	ADA Negative
N	6	129
Change in mNIS+7[a] from baseline relative to placebo[a]	-30.85	-32.32

[a] Change from baseline to Month 18 in mNIS+7 difference between patisiran treatment and placebo treatment;
Source: Study ALN-TTR02-004; Module 5.3.5.1

Reviewer comment: *Approximately 4% of patients who were on patisiran therapy showed detectable ADA status. The titer of ADA was low and the appearance of ADA was reported to be transient. Immunogenicity did not seem to affect the systemic exposures, TTR lowering effects and clinical efficacy of patisiran-LNP in these limited number of ADA positive subjects.*

4.6 Pharmacogenomics

Approximately, 100 mutations in the *TTR* gene are known to cause hATTR-PN. The V30M mutation is the most common mutation to cause hATTR-PN. In the U.S., approximately 40% of subjects with hATTR-PN have the V30M mutation. The Applicant enrolled a total of 39 different *TTR* mutations in their pivotal trial (APOLLO). In addition, the Applicant stratified randomization for the V30M mutation (early onset < 50 years old). Patients with the V30M mutation encompassed 43% of subjects enrolled in APOLLO while patients with early onset V30M encompassed 10.2% of patients. The efficacy findings were not significantly different based on the presence or absence of the V30M mutation with early onset hATTR-PN.

Reviewer Comment: *The antisense stand of patisiran binds in a complementary fashion to the 3' UTR of the TTR mRNA. Almost all reported TTR mutations occur in the coding region. Patisiran is able to decrease the synthesis of both wild type and mutant TTR proteins. Hence, any pharmacogenomic variation in target sequence is unlikely to impact the safety or efficacy of patisiran.*

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

VENKATESWARAN CHITHAMBARAM PILLAI
05/18/2018

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

NDA/BLA #:	210,922
Drug Name:	Onpattro (patisiran) injection 2mg/ml
Indication(s):	Treatment of hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis)
Applicant:	Alnylam Pharmaceuticals Inc.
Date(s):	Submission date: 12/11/17
	PDUFA Date: 8/11/2018
Review Priority:	Priority Review
Biometrics Division:	Division of Biometrics I
Statistical Reviewer:	Xiang Ling, Ph.D.
Concurring Reviewers:	Kun Jin, Ph.D., Team Leader
	Jim Hung, Ph.D., Director
Medical Division:	Division of Neuropharm
Clinical Team:	Rainer Paine, M.D.
	Nicholas Kozauer, M.D., Team Leader
Project Manager:	Anhtu Nguyen

Table of Contents

LIST OF TABLES

1 EXECUTIVE SUMMARY

This is the statistical review of the Alnylam Pharmaceuticals' application for the approval of the investigational drug Onpattro (patisiran) injection. The submitted data overall provided statistical evidence to support the efficacy of patisiran in treating patients with hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis).

The primary efficacy analysis from the pivotal study ALN-TTR02-004 (APOLLO) demonstrated a statistically significant improvement in Modified Neurological Impairment Score +7 (mNIS+7) at 18 months of treatment for patients in the patisiran group compared to placebo (-34 points, $p=9.3\times10^{-24}$). At Month 18, the placebo group showed a worsening of neuropathy of +28 points from baseline while the patisiran group had an improvement of -6 points. The treatment effect was consistent among the subgroups by age, gender, race, and region.

Patisiran showed statistically significant improvement on all key secondary endpoints including: neuropathy symptom specific quality of life (Norfolk QoL-DN), motor strength (NIS-W), disability (R-ODS), gait speed (10-MWT), nutritional status (mBMI) and autonomic symptoms (COMPASS 31) at 18 months.

2 INTRODUCTION

2.1 Overview

A single clinical study, ALN-TTR02-004 (APOLLO), conducted under IND 117395, was intended to provide substantial evidence of efficacy. The study was a randomized, double-blind, placebo-controlled study of patients with familial amyloid polyneuropathy (FAP). Overall, 225 patients were randomized in this study (148 patisiran group, 77 placebo) in many countries worldwide. The primary efficacy endpoint was change from baseline in the mNIS+7 composite neuropathy impairment score at 18 months. The first key secondary endpoint was Norfolk QoL-DN.

2.2 Data Sources

Materials reviewed for this application include the clinical study reports, raw and derived datasets, SAS codes used to generate the derived datasets and tables, protocols, statistical analysis plans, which are in the following directory: \\CDSESUB1\evsprod\NDA210922\0007.

3 STATISTICAL EVALUATION

3.1 Data and Analysis Quality

This reviewer could trace how the primary endpoint was derived from the raw data. Documentation of statistical analysis methods was included with sufficient details for this reviewer to reproduce the applicant's key efficacy results.

3.2 Evaluation of Efficacy

3.2.1 Study ALN-TTR02-004

3.2.1.1 Study Design and Endpoints

Study ALN-TTR02-004 (APOLLO) was initiated on 23 December 2013, and completed on 17 August 2017. The original global protocol was dated on 15 August 2013. There were 5 amendments to the global protocol and the major changes affecting efficacy assessment occurred early during the trial. The final protocol was dated on 08 September 2015. In the original SAP Version 1.0, it was intended that an interim analysis for sample size re-estimation would be conducted; however, no interim analysis was conducted during the study based on external information. The FDA had reviewed the first two SAP versions dated on 31 March 2015 and 31 May 2017 respectively. The final SAP was dated 23 August 2017, prior to the database lock on 14 September 2017.

Study Design

This was a multicenter, multinational, randomized, double-blind study comparing patisiran to placebo in hATTR patients with symptomatic polyneuropathy. Patients were randomized to receive either 0.3 mg/kg patisiran or placebo in a 2:1 ratio and stratified by

1. Neuropathy Impairment Score (NIS; < 50 vs. ≥ 50),
2. early onset V30M (< 50 years of age at onset) vs. all other mutations (including late onset V30M), and
3. previous tetramer stabilizer use (tafamidis or diflunisal) vs. no previous tetramer stabilizer use.

The planned study size was 200; the actual number randomized in this study was 225. Patients received patisiran or placebo once every 21 days for 78 weeks (18 months). All site personnel were blinded to the study treatment, except the pharmacist and designated site personnel who set-up, dispensed, and prepared the infusion. Patients had efficacy assessments at Screening/Baseline, 9 months, and 18 months. Study personnel performing assessments related to the efficacy endpoints were different from the investigator and other personnel managing the patient, and these study personnel were blinded to the results of any previous assessments and any clinical laboratory results that could potentially unblind them.

At the 9-month time point, if the Clinical Adjudication Committee determined that a patient was exhibiting rapid disease progression (defined as ≥24-point increase in mNIS+7 from baseline

and FAP stage progression relative to baseline), the patient's treating physician would provide the patient with the option of discontinuing study drug and receiving local standard of care treatment for FAP. Patients who discontinued study drug would return for their 18-month efficacy assessment and blinding would be maintained throughout.

Efficacy Endpoints

The primary endpoint was the change from baseline of the Modified Neurological Impairment Score +7 (mNIS+7) at 18 months. The mNIS+7 was a composite measure of neurologic impairment, including the modified NIS (weakness NIS-W, and reflexes NIS-R), nerve conduction studies of 5 attributes (Σ5 NCS), quantitative sensory testing (QST, including two subcomponents of heat pain HP and touch pressure TP), and autonomic assessment through postural blood pressure (BP). Two assessments were performed at each visit; each component contributing to the composite score was the average of the 2 assessments. The mNIS+7 was scored from 0 (no impairment) to 304 points (maximum impairment). The maximum points for each component were listed below.

Assessment Tool	Total Points	Components (maximum points)

Modified NIS+7	304	• NIS-W: Weakness (192)
		• NIS-R: Reflexes (20)
		• Quantitative sensory testing by body surface area including touch pressure (TP) and heat as pain (HP): QST-BSA$_{TP+HP5}$ (80)
		• ∑5 nerve conduction studies (10)
		  • Ulnar compound muscle action potential (ulnar CMAP)
		  • Ulnar sensory nerve action potential (ulnar SNAP)
		  • Sural sensory nerve action potential (sural SNAP)
		  • Tibial compound muscle action potential (tibial CMAP)
		  • Peroneal compound muscle action potential (peroneal CMAP)
		• Postural blood pressure (BP) (2)

The secondary endpoints of the study were the changes from baseline at 18 months for the following assessments:

1. Norfolk Quality of Life-Diabetic Neuropathy (QOL-DN).
2. Motor strength by NIS-W score.
3. Level of disability by the Rasch-built Overall Disability Scale (R-ODS).
4. Functional status by 10-meter walk test speed (10-MWT).
5. Nutritional status by modified body mass index (mBMI).
6. Autonomic symptoms by Composite Autonomic Symptom Score 31(COMPASS-31) total score.

The patient reported QOL-DN ranges from -4 to 136 points. It included 35 questions that were divided into 5 domains: Physical Functioning/Large Fiber (15 items), Activities of Daily Living (ADLs) (5 items), Symptoms (8 items), Small Fiber (4 items), and Autonomic (3 items). Domain scores were calculated as the average scores of non-missing items multiplied by the number of items if at least 50% of the items are non-missing. A domain score was missing if more than

50% of the included items were missing. If the scores for all 5 domains are non-missing, then Total QOL was the sum of scores of the 5 domains; however, if at least 1 of the domains was missing and at least 50% of the items were non-missing, then Total QOL was calculated as 35 times the mean of the non-missing items. Otherwise, Total QOL was deemed as missing.

3.2.1.2 Statistical Methodologies

The efficacy analysis set was based on the modified Intent-to-Treat (mITT) population consisting of all patients who were randomized and received at least 1 dose of patisiran or placebo.

The type I error control for secondary endpoints was achieved by a hierarchical ordering procedure, with the order listed in the previous section.

Primary Analysis

The primary analysis of mNIS+7 score was performed using a restricted maximum likelihood (REML) based Mixed-Effects Model Repeated Measures (MMRM) approach. The model included baseline mNIS+7 score as a continuous covariate and fixed effect terms including treatment arm, visit (Month 9 or Month 18), treatment-by-visit interaction, genotype (V30M vs. non-V30M), age at hATTR symptom onset (< 50; ≥ 50), region (North America, Western Europe, and Rest of World), and previous tetramer stabilizer use (yes vs. no). An unstructured covariance structure was used to model the within-patient errors. The Satterthwaite approximation was used to estimate the degrees of freedom.

Analysis of the Key Secondary Endpoints

Key secondary endpoints were analyzed using an MMRM model similar to the model described for the primary analysis of mNIS+7 while adjusting for baseline value of the endpoint being modeled. For these secondary endpoints (except NIS-W), MMRM model also included baseline NIS (< 50 vs. ≥ 50) as a factor. The MMRM model for NIS-W did not include baseline NIS since baseline NIS-W was included as a covariate in the model.

Handling of Data Collected after Alternative FAP Treatment

For the primary analysis of mNIS+7, Norfolk QOL-DN and NIS-W, the assessments collected after alternative FAP treatment (liver transplant or use of tafamidis or diflunisal for more than 14 days) were treated as missing. The data post alternative FAP treatment were used in sensitivity analyses as specified. For all other efficacy endpoints, data collected post alternative FAP treatment were included in analyses.

Handling of Missing mNIS+7 Total Score

To assess the impact of missing mNIS+7 score data, two main sensitivity analyses were conducted. One was multiple imputation/analysis of covariance (MI/ANCOVA) based on the missing at random (MAR) assumption. The missing data was imputed separately for each treatment arm using a regression procedure. One hundred imputed datasets were generated, each analyzed using an ANCOVA model, and the estimates were combined to produce the inferential results.

Reviewer's note: As the MI/ANCOVA is based on the same assumption of MAR for the primary analysis of MMRM, this sensitivity analysis may not be very useful in checking the sensitivity of the primary analysis to the handling of missing data.

The other sensitivity analysis was performed using Pattern-Mixture model (PMM) to assess the robustness of the primary MMRM results to the possible violation of the MAR assumption. In this analysis, multiple imputation with mixed missing data mechanisms per the status of patients were conducted. Specifically, patisiran patients who had missing data after treatment discontinuation were assumed to follow the trajectory of placebo patients and all patients on both treatment groups who died were imputed with the worst outcome observed in the study.

Handling of Missing Components of mNIS+7

The mNIS+7 total score includes the following components/subcomponents: motor strength (NIS-W), reflexes (NIS-R), sensation (QST, including QST-TP and QST-HP), nerve conduction (Σ5 NCS), and sympathetic nerve autonomic function (postural BP). In the primary derivation of mNIS+7 total score, the "within treatment arm" imputation algorithm was used for the imputation of missing component. At each visit, if a patient has a missing component for mNIS+7, the value was imputed using data from other patients who were on the same treatment arm and who had non-missing data for that component at that visit. In a sensitivity analysis, any such missing value was imputed as the mean value for the component at the visit from all patients (combining placebo and patisiran arms).

Binary Analysis

The percentage of patients with <10-point increase in mNIS+7 composite score from baseline to Month 18 was compared between the two treatment arms using the Cochran-Mantel-Haenszel test (CMH), stratified by genotype (V30M vs. non-V30M). In addition, the percentage of patients with a decrease (change from baseline <0 point) in mNIS+7 total score from baseline to Month 18 was also calculated and analyzed using similar methods. Patients with missing 18-month data were counted in the denominators.

Component Analyses

Component analyses were conducted to assess the consistency of treatment effect on the change from baseline at Month 18 for each component of mNIS+7, including NIS-W, NIS-R, QST, Σ5 NCS, and postural blood pressure BP.

3.2.1.3 Patient Disposition, Demographic and Baseline Characteristics

A total of 225 patients were randomized (148 to the patisiran group and 77 to the placebo group) in 44 study centers from 19 countries. The US contributed the highest number of patients (33 patisiran, 9 placebo). Overall 40 (18%) patients discontinued study treatment early (7% vs. 38% in the patisiran and placebo groups, respectively) and 32 (14%) patients withdrew from the study (7% vs.29% in the patisiran and placebo groups, respectively). The dropout rate in the placebo group was much higher than that in the patisiran group. The most common reasons for withdrawal were patient consent withdrawal, adverse event, and death. A total of 7 patients (1

patisiran, 6 placebo) had rapid disease progression as determined by the Clinical Adjudication Committee (Table 1).

Table 1: Patient Disposition

Disposition	**Placebo (N=77)**	**Patisiran (N=148)**	**Overall (N=225)**
Total number of patients			
Randomized	77	148	225
Treated	77 (100.0)	148 (100.0)	225 (100.0)
Completed treatment[a]	48 (62.3)	137 (92.6)	185 (82.2)
Completed study[b]	55 (71.4)	138 (93.2)	193 (85.8)
Discontinuation of treatment	29 (37.7)	11 (7.4)	40 (17.8)
Primary reason for treatment discontinuation			
Adverse event	7 (9.1)	3 (2.0)	10 (4.4)
Death	4 (5.2)	5 (3.4)	9 (4.0)
Progressive Disease	4 (5.2)	1 (0.7)	5 (2.2)
Physician Decision	2 (2.6)	0	2 (0.9)
Protocol deviation	0	1 (0.7)	1 (0.4)
Withdrawal by subject	12 (15.6)	1 (0.7)	13 (5.8)
Withdrawal from study	22 (28.6)	10 (6.8)	32 (14.2)
Primary reason for study withdrawal			
Adverse Event	6 (7.8)	2 (1.4)	8 (3.6)
Death	4 (5.2)	6 (4.1)	10 (4.4)
Physician decision	1 (1.3)	0	1 (0.4)
Protocol deviation	0	1 (0.7)	1 (0.4)
Withdrawal by subject	11 (14.3)	1 (0.7)	12 (5.3)
Patients with rapid disease progression[a]	6 (7.8)	1 (0.7)	7 (3.1)
Patients who discontinued treatment but completed study	8 (10.4)	1 (0.7)	9 (4.0)
Patients who completed treatment but withdrew from study	1 (1.3)	0	1 (0.4)

[a] Rapid disease progression is defined as patients with a ≥24-point increase from baseline in mNIS+7 and a ≥ 1 level increase from baseline in FAP stage at Month 9 as determined by the Clinical Adjudication Committee.
Source: Table 10 of CSR.

Overall, the 2 treatment groups were comparable regarding demographic characteristics. The mean age was 61 years, 74% were male, and 72% were White or Caucasian. The majority (44%) of patients were from Western Europe (Table 2).

Table 2: Demographic Characteristics

Parameter	Placebo (N=77)	Patisiran (N=148)
Age N(%)		
<65	44 (57.1)	86 (58.1)
65-74	24 (31.2)	53 (35.8)
>=75	9 (11.7)	9 (6.1)
Age Mean (SD) (years)	62.2 (10.8)	59.6 (12.0)
Sex N(%)		
Male	58 (75.3)	109 (73.6)
Female	19 (24.7)	39 (26.4)
Race N(%)		
Asian	25 (32.5)	27 (18.2)
American Indian or Alaskan Native	0	0
Black/African or African American	1 (1.3)	4 (2.7)
White/Caucasian	50 (64.9)	113 (76.4)
Other	0	1 (0.7)
More than One Race	0	2 (1.4)
Missing	1 (1.3)	1 (0.7)
Region N(%)		
North America	10 (13.0)	37 (25.0)
Western Europe	36 (46.8)	62 (41.9)
Rest of World	31 (40.3)	49 (33.1)
Asia	21 (27.3)	23 (15.5)
Central & South America	6 (7.8)	13 (8.8)
Eastern Europe	4 (5.2)	13 (8.8)

Source: Table 12 of CSR.

Baseline characteristics in general were balanced between the two treatment groups (Table 3). The mean years since diagnosis of hATTR amyloidosis with polyneuropathy was 2.5 years, and the majority (72%) of patients were age ≥50 years at symptom onset. The mean baseline NIS was 59 points and the mean baseline mNIS+7 was 79 points. Overall, 10% of patients had early onset V30M, and 53% of patients had previously used a tetramer stabilizer.

Table 3: Baseline Disease Characteristics

Parameter	Placebo (N=77)	Patisiran (N=148)
Years since Diagnosis with hATTR Amyloidosis		
Mean	2.60	2.39
SD	3.24	3.26
Median	1.41	1.34
Min, Max	0.0, 16.5	0.0, 21.0
Age at hATTR Amyloidosis Symptom Onset N(%)		
<50 years	20 (26.0)	42 (28.4)

Parameter	Placebo (N=77)	Patisiran (N=148)
>=50 years	57 (74.0)	106 (71.6)
Baseline NIS		
<50	35 (45.5)	62 (41.9)
>=50 - <100	33 (42.9)	63 (42.6)
>=100	9 (11.7)	23 (15.5)
Baseline NIS Mean (SD)	57.0 (32.0)	60.5 (34.5)
Baseline mNIS+7		
Mean	74.6	80.9
SD	37.04	41.51
Median	71.5	76.9
Min, Max	11.0, 153.5	8.0, 165.0
Genotype		
V30M	40 (51.9)	56 (37.8)
Non-V30M	37 (48.1)	92 (62.2)
Genotype Class		
Early onset V30M (<50 years of age at onset)	10 (13.0)	13 (8.8)
All other mutations (including late onset V30M)	67 (87.0)	135 (91.2)
Previous Tetramer Stabilizer Use		
No	36 (46.8)	70 (47.3)
Yes	41 (53.2)	78 (52.7)
Tafamidis	27 (35.1)	47 (31.8)
Diflunisal	14 (18.2)	31 (20.9)

Source: Table 13 of CSR.

3.2.1.4 Results and Conclusions

3.2.1.4.1 Analyses of the Primary Endpoint

The primary analysis of mNIS+7 score is represented in Table 4. A lower score of mNIS+7 indicates less neurologic impairment. There was a statistically significant improvement in mNIS+7 score at Month 18 for patients in the patisiran group compared to the placebo group (LS mean difference: -34 points, $p = 9.3 \times 10^{-24}$). At 18 months, the patisiran group showed an improvement compared to baseline (LS mean: -6 points) while the placebo group showed a worsening of neuropathy (LS mean: +28 points).

Table 4: mNIS+7 Change from Baseline at Month 18 (mITT Population)

Statistic	Placebo (N=77)	Patisiran (N=148)
N	67	141
LS Mean (SEM)	28.0 (2.6)	-6.0 (1.7)
95% CI	22.8, 33.1	-9.5, -2.6

LS Mean (SEM) Difference (Patisiran - Placebo)	-	-34.0 (3.0)
95% CI	-	-39.9, -28.1
p-value	-	9.3 $x10^{-24}$

SEM: standard error of the mean; CI: confidence interval
A lower mNIS+7 score indicates less impairment.
The MMRM model includes baseline mNIS+7 as covariate and fixed-effect terms including treatment group, visit, treatment-by-visit interaction, genotype, age at hATTR symptom onset, previous tetramer stabilizer use, and region.
Source: Table 16 of CSR, confirmed by this reviewer.

Sensitivity Analyses for Missing Data

Missing mNIS+7 Assessments

A total of 17 patients (7[5%] patisiran, 10[13%] placebo) were missing both Month 9 and Month 18 assessments of mNIS+7, hence not included in the mITT population for the primary analysis. In the sensitivity analyses using MI/ANCOVA and PMM models, post-baseline data for these 17 patients were imputed based on the assumptions of missing at random and missing not at random respectively. Since the amount of missing data in the patisiran group was very limited, the results of both analyses were consistent with the primary MMRM analysis (Table 5). In fact, the analyses tend to underestimate the treatment effect as placebo group had much larger dropout rate, at least for usefulness of the drug.

Censored mNIS+7 Assessments

The mNIS+7 assessments were not used in the primary analysis for 8 patients (7 placebo, 1 patisiran) due to the use of alternative treatment for hATTR amyloidosis. Analysis including mNIS+7 data post-alternative treatment yielded similar results to the primary analysis (Table 5).

Missing mNIS+7 Components

The mNIS+7 score included the following components: NIS-W, NIS-R, QST-HP and QST-TP, Σ5 NCS, and postural BP. The missing values for the components at each visit were imputed using data from other patients who were on the treatment arm in the primary analysis, and using data from all patients (combined arms) in the sensitivity analysis. As the amount of missing components was very limited (<1%), the sensitivity resulted in a consistent estimate of the treatment effect on mNIS+7 with the primary analysis (Table 5).

Table 5: Sensitivity Analyses of Change from Baseline mNIS+7 Score at Month 18

Statistic	Placebo (N=77)	Patisiran (N=148)
Multiple Imputation/ANCOVA		
LS Mean (95% CI)	28.6 (22.8, 34.5)	-6.1 (-9.9, -2.4)
LS Mean (95% CI) Difference	-	-34.8 (-41.2, -28.3)
Pattern-Mixture Model		
LS Mean (95% CI)	30.7 (24.6, 36.8)	-3.1 (-7.16, 1.0)
LS Mean (95% CI) Difference	-	-33.8 (-40.55, -26.95)
MMRM Including mNIS+7 Scores Post-Alternative Treatment		
LS Mean (95% CI)	27.0 (22.1, 32.0)	-6.0 (-9.4, -2.6)
LS Mean (95% CI) Difference	-	-33.0 (-38.7, -27.3)

Statistic	Placebo (N=77)	Patisiran (N=148)
Imputing Missing Components Using Combined Arms		
LS Mean (95% CI)	27.6 (22.5, 32.8)	-6.0 (-9.5, -2.6)
LS Mean (95% CI) Difference		-33.7 (-39.5, -27.8)

Source: Table 19 of CSR.

The QST was a component of mNIS+7 that measures sensory neuropathy impairment. It was comprised of measures evaluating touch pressure by body surface area (QST-TP, large nerve fiber sensation) and heat pain by body surface area (QST-HP, small nerve fiber sensation). At each assessment, QST was performed along one side of the body at up to 10 anatomical sites, and each site was assigned a score of 0 (normal), 1 or 2 (maximal abnormality). The scores for QST-HP and QST-TP were calculated as the sum of scores for all anatomical sites. A testing algorithm was predetermined, and once a site was tested as normal, sites proximal to that site were assumed to be normal and not tested. Thus, all patients were tested for foot, leg, hand and forearm, but there were high percentages of patients who were not tested for the remaining 6 sites, including lower abdomen, upper abdomen, deltoid, face, subclavicle, and thigh. The percentages of not evaluated patients for each site were comparable between the two groups at baseline, but were getting higher for the patisiran group and lower in placebo group post baseline (Table 6).

Table 6: Percentage of Patients Not Evaluated for QST Body Sites

	Placebo			Patisiran		
	Baseline	Month 9	Month 18	Baseline	Month 9	Month 18
QST-HP heat pain by body surface area						
Abdomen, Lower	64	55	55	59	68	69
Abdomen, Upper	75	72	65	72	81	85
Deltoid	48	40	39	52	57	60
Foot	0	0	0	0	0	0
Face	65	60	57	62	71	77
Forearm	0	0	0	0	0	0
Hand	0	0	0	0	0	0
Leg	0	0	0	0	0	0
Subclavicle	78	75	69	74	82	86
Thigh	38	28	33	36	40	43
QST-TP touch pressure by body surface area						
Abdomen, Lower	62	54	49	57	58	62
Abdomen, Upper	81	75	71	76	82	82
Deltoid	43	34	45	46	55	57
Foot	0	0	0	0	0	0
Face	75	75	71	70	74	79
Forearm	0	0	0	0	0	0
Hand	0	0	0	0	0	0
Leg	0	0	0	0	0	0

	Placebo			Patisiran		
	Baseline	Month 9	Month 18	Baseline	Month 9	Month 18
Subclavicle	90	82	73	82	87	89
Thigh	31	25	22	24	30	34
Average	37	34	33	36	39	41

Note: percentages were based on the number of patients who took the QST at each visit.

Source: FDA reviewer.

This QST testing algorithm was based the assumption that sensation loss is nerve fiber-length dependent, as was the case in 71% of patients with hATTR amyloidosis with polyneuropathy (per the applicant's response on 5/9/2018 to the review's request for information). To check the impact of this assumption, this reviewer conducted sensitivity analyses by excluding the 12 QST items that had missing data, or by excluding the QST component altogether from the total mNIS+7 score. The analyses yielded smaller but still statistically significant treatment differences (-26.5 and -20.4 points respectively; Table 7).

Table 7: Sensitivity Analyses for Handling Not Tested mNIS+7 Items

Statistic	Placebo (N=77)	Patisiran (N=148)
Excluding 12 QST Items		
LS Mean (95% CI)	24.1 (19.8, 28.3)	-2.4 (-5.3, 0.4)
LS Mean (95% CI) Difference		-26.5 (-31.4, -21.6)
p-value		2.9×10^{-21}
Excluding All QST Items		
LS Mean (95% CI)	20.5 (16.6, 24.5)	0.2 (-2.5, 2.8)
LS Mean (95% CI) Difference		-20.4 (-24.9, -15.8)
p-value		4.4×10^{-16}

Source: FDA reviewer.

Additional Analyses for mNIS+7 score

At Month18, an improvement was observed for patisiran compared to placebo across all the mNIS+7 components. The largest component NIS-W was one of the secondary endpoint and the result was in Table 10. The QST was the secondary largest component. This reviewer also conducted an analysis of the QST items for the body surface areas of foot, leg, hand and forearm that was evaluated for all patients. Both results demonstrated an improvement of patisiran compared to placebo in QST (Table 8).

Table 8: Analyses of QST Component Items for the Body Surface Areas of Foot, Leg, Hand and Forearm

Statistic	Placebo (N=77)	Patisiran (N=148)
Total QST (80 points max possible score)		
LS Mean (95% CI)	7.0 (4.1, 9.9)	-6.0 (-8.0, -4.1)
LS Mean (95% CI) Difference		-13.1 (-16.3, -9.8)
QST Items for the Body Surface Areas of Foot, Leg, Hand and Forearm (48 points max possible score)		
LS Mean (95% CI)	3.2 (1.8, 4.6)	-2.6 (-3.5, -1.7)
LS Mean (95% CI) Difference		-5.8 (-7.4, -4.2)

Source: FDA reviewer.

Binary analyses of the proportion of patients with a change from baseline in mNIS+7 of <0 and <10 points, respectively, also supported the robustness of the primary analysis (results not shown in this review).

Significant issues were identified by inspection at Site #061 in Spain. Analysis excluding this site yielded a similar estimate of treatment difference of -33 points as the primary analysis.

3.2.1.4.2 Analyses of the Secondary Endpoints

The Norfolk QoL-DN is the first key secondary endpoint, including 35 items divided into 5 domains. A lower value represents higher quality of life. The analysis of Norfolk QoL-DN score is represented in

Table 9. There was a statistically significant improvement in Norfolk QoL-DN score at Month 18 for patients in the patisiran group compared to the placebo group (LS mean difference: -21 points, $p = 1.1 \times 10^{-10}$). At Month 18, the patisiran group showed an improvement in quality of life compared to baseline (LS mean: -7 points) while the placebo group showed a worsening of quality of life (LS mean: +14 points).

Table 9: Norfolk QoL-DN Change from Baseline at Month 18

Statistic	Placebo (N=77)	Patisiran (N=148)
N	65	141
LS Mean (SEM)	14.4 (2.73)	-6.7 (1.77)
95% CI	9.0, 19.8	-10.2, -3.3
LS Mean (SEM) Difference (Patisiran - Placebo)	-	-21.1 (3.10)
95% CI	-	-27.2, -15.0
p-value	-	1.1×10^{-10}

A lower Norfolk QoL-DN score indicates higher quality of life.
MMRM model includes baseline Norfolk QoL-DN score as covariate and fixed-effect terms including treatment group, visit, treatment-by-visit interaction, baseline NIS, genotype, age at hATTR symptom onset, previous tetramer stabilizer use, and region.
Source: Table 22 of CSR, confirmed by this reviewer.

There were 12 patients in the placebo group and 7 patients in the patisiran group who did not have evaluable post-baseline Norfolk QoL-DN assessments, and therefore did not contribute to the analysis. Sensitivity analysis using Multiple Imputation/ANCOVA yielded a similar treatment difference. There were no missing domains and very limited missing items (<1%), which had minimal impact on the analyses.

The analyses of the other key secondary endpoint are represented in Table 10. The NIS-W is a component of the primary endpoint mNIS+7, measuring motor strength. The total possible score is 192 points. A lower NIS-W score represents better motor strength. There was a statistically significant improvement in motor strength at Month 18 for patients in the patisiran group compared to the placebo group (LS mean difference: -17.9 points, $p = 1.4 \times 10^{-13}$).

The R-ODS is a patient-reported measure of level of disability on a scale of 0-48, with 0 being the worst and 48 the best. There was a statistically significant improvement in disability at Month 18 for patients in the patisiran group compared to the placebo group (LS mean difference: 9.0 points, $p = 4.1 \times 10^{-16}$).

The 10- meter walk test (10-MWT) is a measure of ambulatory ability and gait speed. There was a statistically significant improvement in 10-MWT gait speed at Month 18 for patients in the patisiran group compared to the placebo group (LS mean difference: 0.31 m/s, $p = 1.9 \times 10^{-12}$).

The mBMI is a measure of nutritional status, calculated as the product of BMI multiplied by the concentration of serum albumin. A lower value indicates worse nutritional status. There was a statistically significant improvement in nutritional status at Month 18 for patients in the patisiran group compared to the placebo (LS mean difference: +115.7 $kg/m^2 \times$albumin g/L, $p = 8.8 \times 10^{-11}$).

The COMPASS 31 is a measure of autonomic neuropathy symptoms on a scale of 0 to 100, with a lower COMPASS 31 represents better autonomic neuropathy symptoms. There was a statistically significant improvement in COMPASS 31 at Month 18 for patients in the patisiran group compared to the placebo (LS mean difference: -7.5 points, p =0.0008).

Table 10: Analyses of the Other Key Secondary Endpoint

Endpoint	Change from Baseline at Month 18 LS Mean (SEM)		Patisiran - Placebo Treatment Difference LS Mean (95% CI)	p-value
	Patisiran	Placebo		
NIS-W[a]	0.05 (1.3)	17.9 (2.0)	-17.9 (-22.3, -13.4)	1.4×10^{-13}
R-ODS[b]	0.0 (0.6)	-8.9 (0.9)	9.0 (7.0, 10.9)	4.1×10^{-16}
10-MWT (m/sec)[b]	0.08 (0.02)	-0.24 (0.04)	0.31 (0.23, 0.39)	1.9×10^{-12}
mBMI[b]	-3.7 (9.6)	-119 (14.5)	116 (82, 149)	8.8×10^{-11}
COMPASS 31[a]	-5.3 (1.3)	2.2 (1.9)	-7.5 (-11.9, -3.2)	0.0008

[a] A lower number indicates less impairment/fewer symptoms.
[b] A higher number indicates better condition.
Source: Table 25-29 of CSR, confirmed by this reviewer.

3.3 Evaluation of Safety

Please see the clinical review.

4 FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

4.1 Gender, Race, and Age

Subgroup analyses showed consistent treatment effect on mNIS+7 across all subgroups by age, gender, race, and region (Table 11).

Table 11: Subgroup Analyses of the Primary Endpoint of mNIS+7

Subgroup	Baseline N, Mean		Change from Baseline at Month 18 LS Mean (SEM)		Patisiran - Placebo Treatment Difference LS Mean (95% CI)
	Placebo	Patisiran	Placebo	Patisiran	
<65	44, 70	86, 74	24.3 (3.1)	-6.3 (2.1)	-30.6 (-38.0, -23.3)
>=65	33, 80	62, 91	36.3 (4.1)	-2.3 (2.7)	-38.5 (-48.3, -28.8)
Male	58, 78	109, 82	29.5 (2.8)	-5.6 (1.9)	-35.1 (-41.8, -28.4
Female	19, 65	39, 77	29.5 (5.5)	-2.2 (3.4)	-31.7 (-44.6, -18.8)
White	50, 73	113, 81	27.9 (3.0)	-6.0 (1.8)	-33.9 (-40.7, -27.1)
Non-White	26, 76	33, 83	31.9 (5.0)	-1.9 (4.4)	-33.7 (-46.5, -21.0)
North America	10, 86	37, 66	43.3 (7.2)	-3.6 (3.5)	-46.9 (-62.8, -31.1)
Western Europe	36, 75	62, 88	28.7 (3.6)	-8.1 (2.5)	-36.8 (-45.4, -28.2)
Rest of World	31, 70	49, 83	26.4 (4.2)	-1.3 (3.3)	-27.7 (-37.9, -17.5)

A lower mNIS+7 score indicates less impairment.
Source: FDA reviewer.

5 SUMMARY AND CONCLUSIONS

5.1 Statistical Issues

In the single pivotal Study APOLLO, there was a much higher percentage of patients in the placebo group who discontinued study treatment (38%) and/or withdrew earlier from the study (29%), compared to the patisiran group (both rates were 7%). However, this would not impact the study conclusion as analyses tend to underestimate the treatment effect under this scenario, at least for usefulness of patisiran.

There were high percentages of patients who were not tested for certain anatomical sites of the QST at each visit, as a result of following a predetermined algorithm. Essentially, a score of 0 (the best score indicating normal condition) was assigned for the anatomical sites that were not tested, based on clinical assumptions. This procedure was prespecified and did not appear to affect the study conclusion.

5.2 Collective Evidence

The primary efficacy analysis demonstrated a statistically significant improvement in mNIS+7 score at Month 18 for patients in the patisiran group compared to the placebo group (LS mean difference: -34 points, $p = 9.3 \times 10^{-24}$). The treatment effect was robust and consistent across subgroups.

There was a statistically significant improvement in the first key secondary endpoint of Norfolk QoL-DN score at Month 18 for patients in the patisiran group compared to the placebo group (LS mean difference: -21 points, $p = 1.1 \times 10^{-10}$). Statistically significant treatment effect was also demonstrated in motor strength by NIS-W score, level of disability by R-ODS, functional status by 10-meter walk test speed, nutritional status by mBMI, and autonomic symptoms by COMPASS-31 total score.

5.3 Conclusions and Recommendations

The data overall provide statistical evidence to support the efficacy of patisiran in improving a series of motor, sensory and autonomic neuropathy clinical endpoints in patients with hATTR amyloidosis compared to placebo at 18 months of treatment.

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/s/

XIANG LING
05/16/2018

KUN JIN
05/16/2018
I concur with the review.

HSIEN MING J HUNG
05/16/2018

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

CARCINOGENICITY STUDIES

NDA/BLA #:	NDA 210,922 (SDN-5; SN0005)
Drug Name:	ALN-TTR02 (patisiran; Onpattro)
Indication(s):	Treatment of adults with hereditary transthyretin-mediated amyloidosis
Applicant:	Alnylam Pharmaceuticals Inc.
Date(s):	November 15, 2017 (Submitted/Received)
Study Reviewed:	A 26-week Intravenous Injection Carcinogenicity Study in TgRasH2 Mice
Biometrics Division:	Division of Biometrics VI
Primary Reviewer:	Eiji Ishida, MS
Concurring Reviewers:	Karl Lin, PhD, Team Leader
Medical Division:	Division of Neurology Products (DNP)
Reviewing Pharmacologist:	David Carbone, PhD
Keywords:	Carcinogenicity, Dose response, Mortality

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1 SUMMARY

The Sponsor conducted a 26-week Intravenous Injection Carcinogenicity Study in TgRasH2 Mice to determine the carcinogenic potential of patisiran (ALN-TTR02), a lipid nanoparticle incorporating a siRNA that is targeted against transthyretin (TTR), when given by intravenous injection once every two weeks for 26 weeks (total of 14 doses) to TgRasH2 hemizygous mice.

The reviewer analyzed the dose-response relationship of tumor incidence and mortality (including tumor-related mortality). The analyses of tumor data consist of analyses for dose-response relationships in tumor incidence for the four dose groups (0, 0.5, 2 and 6 mg/kg), and pairwise comparisons in tumor incidence between each of the treated groups (0.5, 2 and 6 mg/kg) and the vehicle control group (0 mg/kg). A pairwise comparison of the positive control (MNU) to the vehicle control was also conducted.

The Sponsor's *Statistical Analysis of Mortality and Tumor Data (Appendix 17 of the study report)* states that 'In conclusion, for both sexes, the statistical results did not reveal any test item dose-related increase in tumors incidence.' The Sponsor's study report (Summary section) states that that 'Administration of ALN-TTR02 was not associated with any findings indicative of hematological malignancy.'

Statistical Reviewer's Conclusion: This reviewer agrees to the Sponsor's conclusion. The submitted study did not show evidence suggesting that an administration of patisiran (ALN-TTR02) by intraveneous injection once every two weeks to hemizygous TgRasH2 mice for 26 weeks at the three doses (0.5, 2 and 6 mg/kg) was carcinogenic.

2 BACKGROUND

2.1 Main Study Design Elements

In this study, twenty-five male and female TgRasH2 (CByB6F1-Tg(HRAS)2Jic) hemizygous mice were administered saline vehicle control (Group 1) or patisiran at 0.5, 2, or 6 mg/kg (Groups 2, 3, and 4, respectively) every other week [need to figure out mg/kg/day] by IV bolus injection to the tail vein for a total of 14 doses. A positive control group (Group 5) received a single intraperitoneal dose of MNU (N-Methyl-N-Nitrosurea) on Day 1 at 75 mg/kg. There were 25 mice/sex/group. They were 9 weeks old at the initiation of dosing. The saline vehicle control, patisiran, and MNU were administered at a dose volume of 10 mL/kg.

2.2 Submitted Data and Reports

The submission includes one mouse (male and female) study, titled "26-week Intravenous Injection Carcinogenicity Study in TgRasH2 Mice."

The report of this study was submitted in eCTD SN 0005 (SDN-5) on November 15, 2017, and is located at the FDA server:

\\CDSESUB1\evsprod\NDA210922\0005\m4\42-stud-rep\423-tox\4234-carcigen\42342-smt-stud\ttr02-glp15-024

The “tumor.xpt” dataset of the study data was submitted in the same submission. It is located at the FDA server:
\\CDSESUB1\evsprod\NDA210922\0005\m4\datasets\ttr02-glp15-024\tabulations\send

2.3 Statistical Method to Evaluate Carcinogenicity

2.3.1 Survival Analysis

In the reviewer’s analysis of survival data of the mouse carcinogenicity study, the survival distributions of animals in all treatment groups are estimated using the Kaplan-Meier product limit method. For control, low, mid, and high dose groups, a dose response relationship is tested using the likelihood ratio test, and the homogeneity of survival distributions is tested using the log-rank test. The term "dose response relationship" refers to a linear component of treatment effect, and not necessarily a strictly increasing or decreasing mortality or tumor incidence rate as dose increases.

2.3.2 Tumor Data Analysis

Poly-k test: A dose response relationship and pairwise comparisons of each of the three dose groups to the control group are statistically tested for each of the tumor types of interest via the poly-k method,[1] which uses a fractional weighting scheme for animals that were not at the full risk of tumor development. The reported number as labeled *weighted (mortality adjusted) total number of animals* in the poly-k analysis tables represents the risk set obtained by discounting (weighting fractionally) the risk of every animal if it dies before the terminal sacrifice without having the tumor type being tested. If there are no animals that had no tumor and die before the terminal sacrifice, or if all animals die before terminal sacrifice but develop the tumor type being tested, then the size of the risk set equals the number of randomized animals.

The poly-k test is an extension of Cochran-Armitage (CA) test. The CA test assumes that all animals are at an equal risk of the development of the tumor type being tested, regardless of the time of their death, while the poly-k test assigns a discounted risk of the tumor development to an animal that dies before the terminal sacrifice without having had the tumor type, by applying an appropriate polynomial weight. The weighting scheme of the poly-k method is based on an idea that an animal that dies before the terminal sacrifice without developing the tumor type was under a smaller onset risk of the tumor type. An animal may live till the end of a study period or die before the end of a study period, i.e., the time of the terminal sacrifice. If an animal dies before the terminal sacrifice without having had the tumor of interest, the risk of a tumor onset is considered having been reduced because of its shorter exposure duration. In this review, a polynomial weight of k=3 is used.

Multiple testing adjustment: For the adjustment of multiple testing of dose response relationship alone, the FDA guidance for the carcinogenicity study design and data analysis suggests the use of test levels α=0.005 for a common tumor and α =0.025 for a rare tumor for a submission with

[1] The details of this method are found in the following articles: Bailer and Portier (1988) and Bieler and Williams (1993).

two two-year studies in two species. A rare tumor is defined as one in which the published spontaneous tumor rate or the incidence rate of the tumor type in the concurrent control group is less than 1%. For multiple pairwise comparisons of individual treated groups with control alone, the FDA guidance suggests the use of test levels α=0.01 for a common tumor and α=0.05 for a rare tumor. The use of the recommended test levels of significance will result in an overall false positive rate of approximately 10% for both submissions with two or one species. It should be noted that the FDA guidance for multiple testing for dose response relationship is based on a publication by Lin and Rahman (1998). In this work the authors investigated the use of this rule for Peto analysis. In a later work Rahman and Lin (2008) showed that this rule for multiple testing for dose response relationship is also suitable for poly-k tests.

3 STATISTICAL EVALUATION

3.1 Mouse Study

Table 1 and Table 2 (male/female mice) list all organs recorded in the submitted dataset. The listed organs were those that were examined in the Sponsor's submitted data. This reviewer calculated the numbers of animals *examined and found usable* from the submitted tumor data.

Table 1: Frequency of Tumors (#Examined Animals) in Organ (Male Mice)

Organ	[#Tumors]/[#Animals Examined] BY ORGAN]	0 mg	.5 mg	2 mg	6 mg	Positive Control
body cavity, nasal	0/1	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)
body cavity, thoracic	1/1	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)
bone, sternum	0/124	0 (25)	0 (25)	0 (25)	0 (25)	0 (24)
esophagus	0/124	0 (25)	0 (25)	0 (24)	0 (25)	0 (25)
gallbladder	0/121	0 (25)	0 (23)	0 (25)	0 (24)	0 (24)
galt	0/116	0 (23)	0 (22)	0 (25)	0 (21)	0 (25)
gland, harderian	1/125	0 (25)	0 (25)	1 (25)	0 (25)	0 (25)
gland, mammary	0/0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
gland, parathyroid	0/104	0 (22)	0 (24)	0 (15)	0 (19)	0 (24)
gland, pituitary	0/120	0 (25)	0 (25)	0 (24)	0 (23)	0 (23)
gland, salivary, mandibular	1/125	0 (25)	0 (25)	0 (25)	1 (25)	0 (25)
hemolymphoreticular tissue	18/125	0 (25)	1 (25)	0 (25)	0 (25)	17 (25)
large intestine, rectum	0/123	0 (25)	0 (25)	0 (24)	0 (25)	0 (24)
liver	3/125	1 (25)	0 (25)	1 (25)	0 (25)	1 (25)
lung	14/125	0 (25)	2 (25)	4 (25)	3 (25)	5 (25)
lymph node	0/6	0 (0)	0 (0)	0 (0)	0 (1)	0 (5)
lymph node, mandibular	0/121	0 (25)	0 (24)	0 (22)	0 (25)	0 (25)
lymph node, mesenteric	0/124	0 (25)	0 (25)	0 (25)	0 (24)	0 (25)

Organ	[#Tumors]/[#Animals Examined] BY ORGAN]	0 mg	.5 mg	2 mg	6 mg	Positive Control
muscle, diaphragm	0/1	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)
nerve, optic	0/123	0 (25)	0 (25)	0 (24)	0 (25)	0 (24)
nerve, sciatic	0/124	0 (25)	0 (25)	0 (25)	0 (25)	0 (24)
site, injection	0/99	0 (25)	0 (25)	0 (25)	0 (24)	0 (0)
skin	5/124	0 (25)	0 (24)	0 (25)	0 (25)	5 (25)
spleen	4/125	1 (25)	0 (25)	1 (25)	1 (25)	1 (25)
stomach	12/125	0 (25)	0 (25)	0 (25)	0 (25)	12 (25)
subcutis	1/2	0 (0)	0 (0)	0 (0)	1 (1)	0 (1)
thymus	0/124	0 (25)	0 (25)	0 (24)	0 (25)	0 (25)
ureter	0/2	0 (0)	0 (0)	0 (1)	0 (1)	0 (0)

Note: The frequency of tumors does not always match that of animals, as an animal can have more than one tumor within an organ. The numbers reported in parentheses for each dose group are the counts of examined animals.

Table 2: Frequency of Tumors (#Examined Animals) in Organ (Female Mice)

Organ	[#Tumors]/[#Animals Examined] BY ORGAN]	0 mg	.5 mg	2 mg	6 mg	Positive Control
body cavity, nasal	0/0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
body cavity, thoracic	0/1	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)
brain	1/125	0 (25)	1 (25)	0 (25)	0 (25)	0 (25)
cervix	3/125	0 (25)	1 (25)	0 (25)	0 (25)	2 (25)
esophagus	1/125	0 (25)	0 (25)	0 (25)	0 (25)	1 (25)
gallbladder	0/124	0 (24)	0 (25)	0 (25)	0 (25)	0 (25)
galt	0/120	0 (25)	0 (25)	0 (24)	0 (23)	0 (23)
gland, harderian	1/125	0 (25)	0 (25)	0 (25)	0 (25)	1 (25)
gland, mammary	1/121	0 (23)	0 (24)	0 (25)	0 (25)	1 (24)
gland, parathyroid	0/105	0 (24)	0 (21)	0 (20)	0 (18)	0 (22)
hemolymphoreticular tissue	24/125	1 (25)	1 (25)	0 (25)	0 (25)	22 (25)
kidney	1/125	0 (25)	0 (25)	0 (25)	0 (25)	1 (25)
large intestine, rectum	1/124	0 (25)	1 (25)	0 (25)	0 (25)	0 (24)
liver	1/125	0 (25)	1 (25)	0 (25)	0 (25)	0 (25)
lung	4/125	1 (25)	0 (25)	0 (25)	0 (25)	3 (25)
lymph node	1/16	0 (1)	0 (4)	1 (3)	0 (1)	0 (7)
lymph node, mandibular	0/122	0 (24)	0 (24)	0 (25)	0 (25)	0 (24)
lymph node, mesenteric	1/124	0 (25)	0 (25)	0 (24)	0 (25)	1 (25)
muscle, diaphragm	0/0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
muscle, skeletal	0/124	0 (25)	0 (25)	0 (24)	0 (25)	0 (25)

Organ	[#Tumors]/[#Animals Examined] BY ORGAN]	0 mg	.5 mg	2 mg	6 mg	Positive Control
nerve, optic	0/123	0 (25)	0 (25)	0 (25)	0 (25)	0 (23)
site, injection	0/100	0 (25)	0 (25)	0 (25)	0 (25)	0 (0)
skin	10/125	0 (25)	1 (25)	0 (25)	1 (25)	8 (25)
spleen	10/125	5 (25)	1 (25)	0 (25)	3 (25)	1 (25)
stomach	11/125	1 (25)	0 (25)	0 (25)	0 (25)	10 (25)
subcutis	0/2	0 (0)	0 (0)	0 (0)	0 (1)	0 (1)
thymus	0/124	0 (24)	0 (25)	0 (25)	0 (25)	0 (25)
trachea	0/121	0 (25)	0 (25)	0 (24)	0 (23)	0 (24)
ureter	0/0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
uterus	7/125	0 (25)	0 (25)	0 (25)	0 (25)	7 (25)
vagina	1/125	0 (25)	0 (25)	0 (25)	0 (25)	1 (25)

Note: The frequency of tumors does not always match that of animals, as an animal can have more than one tumor within an organ. The numbers reported in parentheses for each dose group are the counts of examined animals.

3.1.1 Survival Analysis

Sponsor's Mortality Analysis

The Sponsor's study report[2] states: "Compared with the saline control group, the intravenous injection of ALN-TTR02 once every two weeks at doses up to 6 mg/kg for 26 weeks had no effect on survivability during the course of this study." The Sponsor adds that:

> The survival rate was high in males and females of the saline control and ALN-TTR02 groups ranging from 88-100%. These results contrasted with the low survival rate of 40% and 16% noted in males and females in the positive control group (MNU), respectively. Lymphoma was the most frequent cause of death in males and females of the positive control group (MNU). Squamous cell carcinoma and/or hemangiosarcoma were the two most frequent neoplasms as cause of death in the saline control and ALN-TTR02 groups. The cause of death was undetermined in a few mice and miscellaneous other neoplastic and non-neoplastic lesions were considered the cause of death in the remaining mice.

The Sponsor's survivability analysis results are summarized in the table below. This reviewer confirmed the Sponsor's reported analysis results (see The Kaplan-Meier curves for survival rates are given in Figure 1 and Figure 2 of Appendix for male and female mice, respectively. The intercurrent mortality data are given Table 4 and Table 6 for male and female mice, respectively. The results of the tests for dose response relationship and homogeneity of survival are given in Table 5 and Table 7 for male and female rats, respectively.

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[2] Section 10 (pp 32-33) of the study report

Table 3: Sponsor Survivability Analysis

	Male					Female				
Group Dose (mg/kg)	1 0[a]	2 0.5	3 2	4 6	5 75[b]	1 0[a]	2 0.5	3 2	4 6	5 75[b]
Survivability	25/25	25/25	22/25	23/25	10/25	22/25	23/25	23/25	23/25	4/25
Survival rate	100%	100%	88%	92%	40%	88%	92%	92%	92%	16%
Cause of Death										
Lymphoma	0	0	0	0	14	1	0	0	0	20
Hemangiosarcoma	0	0	1	1	0	0	0	1	1	0
Squamous cell carcinoma	0	0	0	0	0	1	1	0	0	1
Accidental	0	0	0	0	0	0	1	0	0	0
Undetermined	0	0	0	0	1	1	0	0	1	0

[a] Saline
[b] N-Methyl-N-Nitrosourea (MNU); positive control

[Source: Text Table 11 (Section 10.2 of Sponsor's study report)]

Reviewer's Survival Analysis

The Kaplan-Meier curves for survival rates are given in Figure 1 and Figure 2 of Appendix for male and female mice, respectively. The intercurrent mortality data are given Table 4 and Table 6 for male and female mice, respectively. The results of the tests for dose response relationship and homogeneity of survival are given in Table 5 and Table 7 for male and female rats, respectively.

Table 4: Intercurrent Mortality Rate (Male Mice)

	0 mg|kg (Control) N=25		0.5 mg|kg (Low Dose) N=25		2 mg|kg (Medium Dose) N=25		6 mg|kg (High Dose) N=25		75 mg|kg (Positive Control) N=25	
Week	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
1 - 13	.	.	.	.	.	.	.	.	2	8.00
14 - 26	.	.	.	.	3	12.00	2	8.00	13	60.00
Ter. Sac.	25	100.00	25	100.00	22	88.00	23	92.00	10	40.00

Table 5: Intercurrent Mortality Comparison (Male Mice)

Test	0, 0.5, 2, 6 mg|kg (Control, Low, Mid, High)	0 vs. 0.5 mg|kg (Control vs. Low)	0 vs 2 mg|kg (Control vs. Med)	0 vs. 6 mg|kg (Control vs. High)	Control vs Positive Comtrol
	p values				
Dose Response (Likelihood Ratio)	0.1792	-	0.0384	0.0935	<0.0001
Homogeneity (Log Rank)	0.1296	-	0.0770	0.1531	<0.0001

Table 6: Intercurrent Mortality Rate (Female Mice)

	0 mg|kg (Control) N=25		0.5 mg|kg (Low Dose) N=25		2 mg|kg (Medium Dose) N=25		6 mg|kg (High Dose) N=25		75 mg|kg (Positive Control) N=25	
Week	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
1 - 13	.	.	1	4.00	.	.	1	4.00	1	4.00
14 - 26	3	12.00	1	8.00	2	8.00	1	8.00	20	84.00
Ter. Sac.	22	88.00	23	92.00	23	92.00	23	92.00	4	16.00

Table 7: Intercurrent Mortality Comparison (Female Mice)

Test	0, 0.5, 2, 6 mg|kg (Control, Low, Mid, High)	0 vs. 0.5 mg|kg (Control vs. Low)	0 vs 2 mg|kg (Control vs. Med)	0 vs. 6 mg|kg (Control vs. High)	Control vs Positive Comtrol
	p values				
Dose Response (Likelihood Ratio)	0.6462	0.6740	0.6607	0.6462	<0.0001
Homogeneity (Log Rank)	0.6458	0.3301	0.6605	0.6458	<0.0001

Findings: As seen from Table 4 and Table 6, the numbers (proportions) of death before terminal sacrifice was 0 (0.0%), 0 (0.0%), 3 (12.0%), 2 (8.0%) and 15 (60.0%) in male mice and 3 (12.0%), 2 (8.0%), 2 (8.0%), 2 (8.0%) and 21 (84.0%) in female mice in the Control, Low dose, Medium dose, High dose and Positive Control groups, respectively. For both male and female mice, as seen in Table 5 and Table 7, at the significance level of 5% (two-sided), the statistical tests did not show a statistically significant dose response relationship in mortality across Control and the three patisiran-treated groups, and the pairwise comparisons did not show a statistically significant mortality between Control and each of the three patisiran-treated groups for male and female. In the pairwise comparison of Positive Control with Vehicle Control, the statistical tests showed statistical significance at the significance level of 5% (two-sided) for both male and female mice.

Sponsor's Tumor Data Analysis

The Sponsor states:

> In conclusion, for both sexes, the statistical results did not reveal any test item dose-related increase in tumors incidence. However, the interpretation of the results should be accomplished with the consideration of both historical control data and biological relevance. In fact, the evaluation of the carcinogenic potential of the test item should be undertaken in the light of an overall scientific judgment.

The Sponsor's analysis plan and results are described as follows:

> For each dataset of interest within each sex, the significance of an overall linear dose-related increase in tumor incidence, across saline Group 1 (saline vehicle) and each of the three test item treated Groups 2, 3, and 4 (0.5, 2.5 and 6 mg/kg) was evaluated, at the 5%

significance level, via Cochran-Armitage's one-sided exact test. The results of this test revealed no significance, at the 5% level, for both sexes.

Furthermore, saline Group 1 was compared to each of the three test item treated Groups 2, 3, 4, and to positive control Group 5 (positive control). These pairwise comparisons were implemented, at the 5% significance level, via Fisher's exact one-sided test in order to check if the tumor incidence rate in each of Groups 2, 3, 4, and 5 is significantly higher than the tumor incidence rate in Group 1. The statistical results revealed that the incidence rate in positive control Group 5 is significantly higher than the one in saline Group 1 for the tumors.

The Sponsor's main tumor findings[3] are listed from the report as shown below.

Table 8: Main Sponsor Findings: Incidence of Lung Neoplastic Tumor Types

	Males					Females				
Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Dose (mg/kg)	**0**	**0.5**	**2**	**6**	**75**	**0**	**0.5**	**2**	**6**	**75**
No. Animals Examined	25	25	25	25	25	25	25	25	25	25
Lung										
Bronchioloalveolar adenoma	0	2	2	3	4	0	0	0	0	3
Bronchioloalveolar carcinoma	0	0	2	0	1	1	0	0	0	0
Adenoma + carcinoma	0	2	4	3	5	1	0	0	0	3

Note: Group 1 is Vehicle Control, Groups 2-4 are ALN-TTR02 doses, and Group 5 is Positive Control.
[Source: Text Table 5 Incidence of Lung Neoplastic Findings (from page 1460 of the study report)]

Table 9: Main Sponsor Findings: Incidence of Noteworthy Neoplastic Tumor Types

	Males					Females				
Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Dose (mg/kg)	**0**	**0.5**	**2**	**6**	**75**	**0**	**0.5**	**2**	**6**	**75**
No. Animals Examined	25	25	25	25	25	25	25	25	25	25
Hemolymphoreticular tissue										
Lymphoma, malignant	0	1	0	0	16	1	1	0	0	22
Skin										
Papilloma	0	0	0	0	4	0	0	0	0	4
Squamous cell carcinoma	0	0	0	0	1	0	1	0	1	4
Stomach										
Papilloma	0	0	0	0	12	0	0	0	0	7
Squamous cell carcinoma	0	0	0	0	0	1	0	0	0	3
Spleen										
Hemangiosarcoma	1	0	1	1	1	5	1	0	3	1

Note: Group 1 is Vehicle Control, Groups 2-4 are ALN-TTR02 doses, and Group 5 is Positive Control.
[Source: Text Table 7 Noteworthy Neoplastic Findings (from page 1461 of the study report)]

This reviewer confirmed the results of the above tables the Sponsor provided in the study report (see this reviewer's analysis results, Table 10, Table 11, Table 12 and Table 13 of this review).

[3] Section 4.3.2 Neoplastic Findings of Appendix 15: Pathology of the study report

Reviewer's Tumor Data Analysis

This reviewer analyzed all recorded tumor incidences in his tumor data analysis, and conducted combined tumor analyses based on the Sponsor's plan as well as the pharm-tox reviewer's suggestions. The Sponsor states in Statistical Analysis of Mortality and Tumor Data (Appendix 17 of the study report):

> For the tumor data, the statistical evaluation was limited to subcutis and hemolymphoreticular tissue using all study animals, to all non-secondary neoplastic lesions found in study plan-required tissues/sites, and to the combination of hemangiosarcoma findings across whole body.
>
> In addition, as per the general study note signed on 16 Sep 2016, bronchioloalveolar adenoma and carcinoma listed under lung were combined and the resulting combination, denoted by "adenoma/carcinoma, bronchioloalveolar", was listed under lung and was also statistically analyzed.

Table 10 (Male Mice) and Table 11 (Female Mice) display the numbers of tumor-bearing animals and examined animals with the size of mortality adjusted risk set for tumors by dose group for each sex. They also list p values for trend and pairwise comparison tests based on poly-3 analysis.

Reviewer's Findings: The findings described below are in accordance with the multiplicity adjustment specified in the FDA guidance for the carcinogenicity study design and data analysis.

Male/Female Mice

<u>Dose response relationship</u>

No tumor types tested showed statistically significant trend in tumor incidence.

<u>Pairwise comparisons:</u>

No tumor types tested showed statistically significant pairwise increases in incidence rate in treated groups when compared with the combined control group.

4 CONCLUSIONS

This reviewer agrees to the Sponsor's conclusion. The submitted study did not show evidence suggesting that an administration of patisiran (ALN-TTR02) by intraveneous injection once every two weeks to hemizygous TgRasH2 mice for 26 weeks at the three doses (0.5, 2 and 6 mg/kg) was carcinogenic.

5 APPENDIX

Figure 1: Kaplan-Meier Survival Curves (Male Mice)

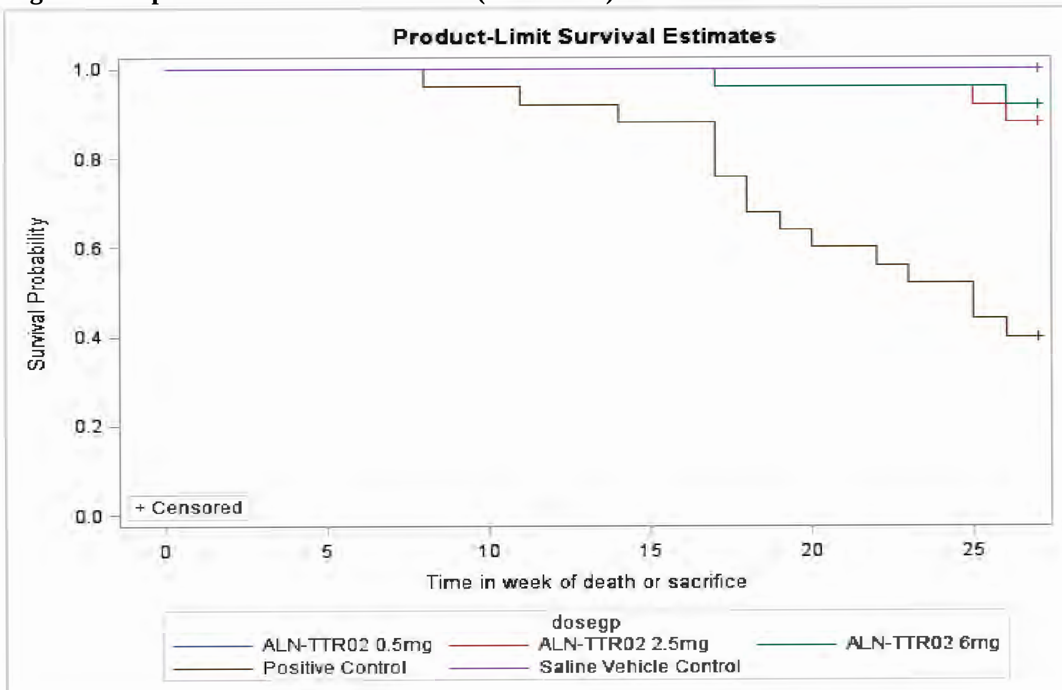

Figure 2: Kaplan-Meier Survival Curves (Female Mice)

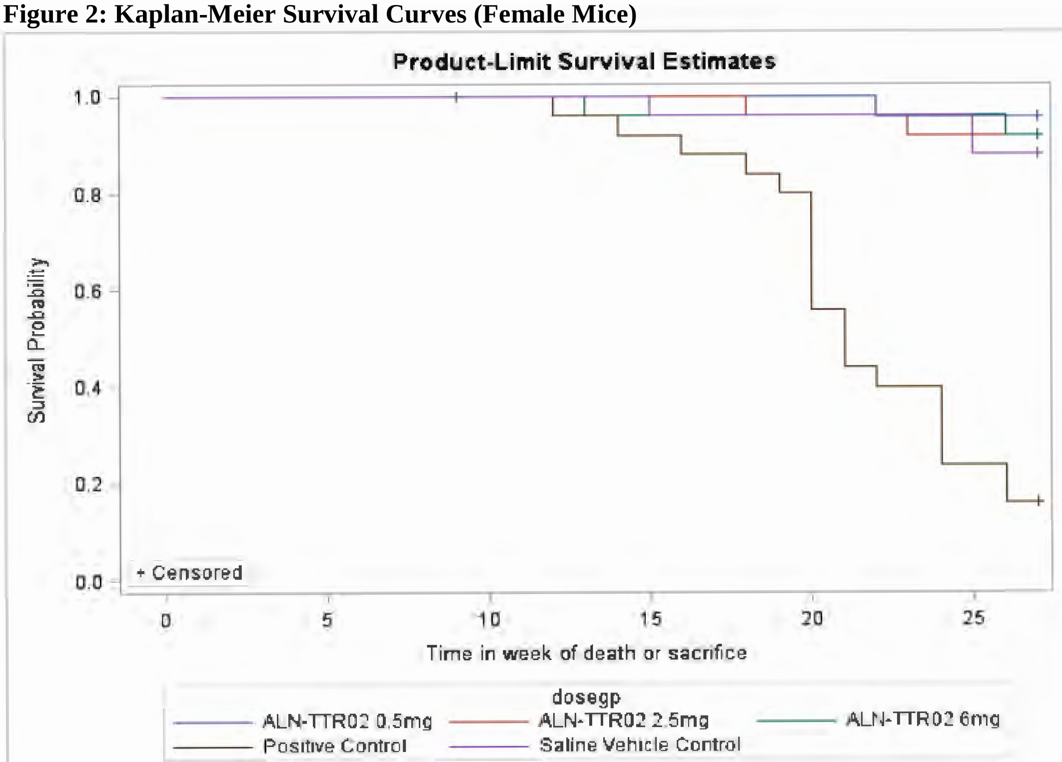

Table 10: P-values for Poly-3 Trend Test and Pairwise Comparisons with Vehicle Control [Sponsor's Tumor Records and Selected Combined Tumor Records] (Male Mice)

Organ	Tumor	Vehicle Control (0 mg)	Low Dose (.5 mg)	Med Dose (2 mg)	High Dose (6 mg)
		Observed Proportion #Animals with Tumor/Total Number of Examined Animals (Poly-3 Mortality Adjusted Total Number of Animals)			
		P value Trend test	Pairwise Comparison with Control		
All sites 1	#Hemangiosarcoma	1/25 (25) 0.1570	0/25 (25) 1.0000	2/25 (24) 0.4844	2/25 (24) 0.4844
All sites 2	#Hemangioma/Hemangiosarcoma	2/25 (25) 0.2740	0/25 (25) 1.0000	2/25 (24) 0.6798	2/25 (24) 0.6798
gland, harderian	adenoma	0/25 (25) 0.4898	0/25 (25)	1/25 (24) 0.4898	0/25 (24)
gland, salivary, mandibular	myoepithelioma, malignant	0/25 (25) 0.2525	0/25 (25)	0/25 (24)	1/25 (25) 0.5000
hemolymphoreticular tissue	lymphoma, malignant	0/25 (25) 0.7449	1/25 (25) 0.5000	0/25 (24)	0/25 (24)
liver	hemangioma	1/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000	0/25 (24) 1.0000
	hepatocellular adenoma	0/25 (25) 0.4898	0/25 (25)	1/25 (24) 0.4898	0/25 (24)
lung	#LUNG bronchioloalveolar Adenoma/Carcinoma	0/25 (25) 0.4898	0/25 (25)	2/25 (24) 0.2347	0/25 (24)
	bronchioloalveolar adenoma	0/25 (25) 0.0873	2/25 (25) 0.2449	2/25 (24) 0.2347	3/25 (24) 0.1099
	bronchioloalveolar carcinoma	0/25 (25) 0.4898	0/25 (25)	2/25 (24) 0.2347	0/25 (24)
spleen	hemangiosarcoma	1/25 (25) 0.3805	0/25 (25) 1.0000	1/25 (24) 0.7449	1/25 (24) 0.7449

Note: The p values are reported in the second row of each cell. The p value reported in the control column is for Trend test, and the other three p values are for pairwise comparison of each indicated dose group to the control group. If no tumor is found in a dose group, its pairwise comparison is not possible to conduct, and thus the p value is not obtainable. The numerator indicates the number of animals that had a tumor of interest, and the denominator the number of animals examined (and found useful). The number inside of ( ) indicates the weighted (mortality adjusted) total number of animals for the poly-k analysis. # indicates the entry is a combined tumor type.

Table 11: P-values for Poly-3 Trend Test and Pairwise Comparisons with Vehicle Control [Sponsor's Tumor Records and Selected Combined Tumor Records] (Female Mice)

Organ	Tumor	Vehicle Control (0 mg)	Low Dose (.5 mg)	Med Dose (2 mg)	High Dose (6 mg)
		Observed Proportion			
		#Animals with Tumor/Total Number of Examined Animals (Poly-3 Mortality Adjusted Total Number of Animals)			
		P value			
		Trend test	Pairwise Comparison with Control		
All sites 1	#Hemangiosarcoma	5/25 (24) 0.6485	2/25 (24) 0.9514	1/25 (25) 0.9904	3/25 (25) 0.8896
brain	meningioma, malignant	0/25 (24) 0.7500	1/25 (24) 0.5000	0/25 (24)	0/25 (24)
cervix	hemangiosarcoma	0/25 (24) 0.7500	1/25 (24) 0.5000	0/25 (24)	0/25 (24)
hemolymphoreticular tissue	lymphoma, malignant	1/25 (25) 0.9356	1/25 (24) 0.7449	0/25 (24) 1.0000	0/25 (24) 1.0000
large intestine, rectum	squamous cell carcinoma	0/25 (24) 0.7500	1/25 (24) 0.5000	0/25 (24)	0/25 (24)
liver	hepatocellular adenoma	0/25 (24) 0.7526	1/25 (25) 0.5102	0/25 (24)	0/25 (24)
lung	bronchioloalveolar carcinoma	1/25 (24) 1.0000	0/25 (24) 1.0000	0/25 (24) 1.0000	0/25 (24) 1.0000
lymph node	hemangiosarcoma	0/1 (1) 0.4444	0/4 (4)	1/3 (3)	0/1 (1) 0.7500
skin	squamous cell carcinoma	0/25 (24) 0.3132	1/25 (24) 0.5000	0/25 (24)	1/25 (24) 0.5000
spleen	hemangiosarcoma	5/25 (24) 0.5546	1/25 (24) 0.9890	0/25 (24) 1.0000	3/25 (25) 0.8896
stomach	squamous cell carcinoma	1/25 (24) 1.0000	0/25 (24) 1.0000	0/25 (24) 1.0000	0/25 (24) 1.0000

Note: The p values are reported in the second row of each cell. The p value reported in the control column is for Trend test, and the other three p values are for pairwise comparison of each indicated dose group to the control group. If no tumor is found in a dose group, its pairwise comparison is not possible to conduct, and thus the p value is not obtainable. The numerator indicates the number of animals that had a tumor of interest, and the denominator the number of animals examined (and found useful). The number inside of ( ) indicates the weighted (mortality adjusted) total number of animals for the poly-k analysis. # indicates the entry is a combined tumor type.

Table 12: Pairwise Comparison of Vehicle Control with Positive Control [Sponsor's Tumor Records and Selected Combined Tumor Records] (Male Mice)

Organ	Tumor Types	Vehicle Control vs Positive Control (MNU 75 mg/kg)		
		Observed Proportion #Animals with Tumor/Total Number of Examined Animals (Poly-3 Mortality Adjusted Total Number of Animals)		P Value
		Vehicle Control	Positive Control	Pairwise Comparison with Positive Control
All sites 1	#Hemangiosarcoma	1/25 (25)	1/25 (17)	0.6516
All sites 2	#Hemangioma/Hemangiosarcoma	1/25 (25)	0/25 (17)	1.0000
hemolymphoreticular tissue	histiocytic sarcoma	0/25 (25)	1/25 (17)	0.4048
	lymphoma, malignant	0/25 (25)	16/25 (24)	<0.001
liver	hemangioma	1/25 (25)	0/25 (17)	1.0000
	hepatocellular adenoma	0/25 (25)	1/25 (17)	0.4048
lung	#LUNG bronchioloalveolar Adenoma/Carcinoma	0/25 (25)	1/25 (17)	0.4048
	bronchioloalveolar adenoma	0/25 (25)	4/25 (18)	0.0248
	bronchioloalveolar carcinoma	0/25 (25)	1/25 (17)	0.4048
skin	papilloma	0/25 (25)	4/25 (18)	0.0248
	squamous cell carcinoma	0/25 (25)	1/25 (17)	0.4048
spleen	hemangiosarcoma	1/25 (25)	1/25 (17)	0.6516
stomach	papilloma	0/25 (25)	12/25 (19)	<0.001

Note: The p value reported is from a pairwise comparison of Vehicle Control to Positive Control, based on Poly-3 trend analysis. The numerator indicates the number of animals that had a tumor of interest, and the denominator the number of animals examined (and found useful). The number inside of ( ) indicates the weighted (mortality adjusted) total number of animals for the poly-k analysis. # indicates the entry is a combined tumor type.

Table 13: Pairwise Comparison of Vehicle Control with Positive Control [Sponsor's Tumor Records and Selected Combined Tumor Records] (Female Mice)

Organ	Tumor Types	Vehicle Control vs Positive Control (MNU 75 mg/kg) Observed Proportion #Animals with Tumor/Total Number of Examined Animals (Poly-3 Mortality Adjusted Total Number of Animals)		P Value Pairwise Comparison with Positive Control
		Vehicle Control	Positive Control	
All sites 1	#Hemangiosarcoma	5/25 (24)	2/25 (16)	0.8659
	Leiomyosarcoma	0/25 (24)	1/25 (15)	0.3846
	polyp	0/25 (24)	1/25 (16)	0.4000
esophagus	papilloma	0/25 (24)	1/25 (15)	0.3846
gland, harderian	adenoma	0/25 (24)	1/25 (15)	0.3846
gland, mammary	adenocarcinoma	0/23 (22)	1/24 (15)	0.4054
hemolymphoreticular tissue	lymphoma, malignant	1/25 (25)	22/25 (25)	<0.001
kidney	adenoma	0/25 (24)	1/25 (16)	0.4000
lung	#LUNG bronchioloalveolar Adenoma/Carcinoma	1/25 (24)	0/25 (15)	1.0000
	bronchioloalveolar adenoma	0/25 (24)	3/25 (17)	0.0638
	bronchioloalveolar carcinoma	1/25 (24)	0/25 (15)	1.0000
lymph node, mesenteric	hemangiosarcoma	0/25 (24)	1/25 (15)	0.3846
skin	papilloma	0/25 (24)	4/25 (17)	0.0235
spleen	hemangiosarcoma	5/25 (24)	1/25 (16)	0.9649
stomach	papilloma	0/25 (24)	7/25 (17)	<0.001
	squamous cell carcinoma	1/25 (24)	3/25 (16)	0.1670
uterus	adenocarcinoma	0/25 (24)	1/25 (15)	0.3846
	polyp	0/25 (24)	6/25 (17)	0.0028
vagina	polyp	0/25 (24)	1/25 (15)	0.3846

Note: The p value reported is from a pairwise comparison of Vehicle Control to Positive Control, based on Poly-3 trend analysis. The numerator indicates the number of animals that had a tumor of interest, and the denominator the number of animals examined (and found useful). The number inside of ( ) indicates the weighted (mortality adjusted) total number of animals for the poly-k analysis. # indicates the entry is a combined tumor type.

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/s/

EIJI ISHIDA
04/25/2018

KARL K LIN
04/25/2018
Concur with review.

Center for Drug Evaluation and Research
Division of Cardiovascular and Renal Products

DCRP Consult NDA 210922

DATE:	Date of Document: 12/11/2017 Date of Consult: 12/9/2017 Desired Completion Date: 3/8/2018 Date of Completion: 3/13/2018
FROM:	Preston M. Dunnmon, M.D., M.B.A., Medical Officer Division of Cardiovascular and Renal Products, HFD-110
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TO:	Nick Kozauer, MD, CDTL Division of Neurology Products, HFD-120
DRUG NAME:	Onpattro (Patisiran-LPN, ALN-TTR02)
DOSE/FORMULATION:	Lipid nanoparticle formulation (Patisiran-LNP) for IV administration every 3 weeks at 0.3 mg/kg over approximately 80 minutes, to be preceded by premedication with a corticosteroid, acetaminophen and antihistamines
MECH OF ACTION:	A double-stranded small interfering ribonucleic acid (siRNA) targeting a conserved region in the 3' untranslated region (UTR) of wt and mutant TTR mRNA
APPLICANT:	Alnylam Pharmaceuticals

CONSULT QUESTION: "Please comment on the interpretability of the cardiac assessments performed during this development program."

DOCUMENTS REVIEWED (Studies with Cardiac Assessments):

- Clinical Study Report Study ALN-TTR02-002 (Phase 2 MAD, uncontrolled, N=29 patisiran-LPN, 2 doses):
- Clinical Study Report Study ALN-TTR02-003 (OLE, uncontrolled, for subjects from studies 002

- Clinical Study Report Study ALN-TTR02-004 (APOLLO Phase 3, N=148 patisiran-LPN, N=77 placebo, 18 months)
- Clinical Study Report Study ALN-TTR02-006 (Global OLE, uncontrolled, for subjects from studies 002/3 and 004, N=188 patisiran-LNP up to ~5 years dosing).

EXECUTIVE SUMMARY: Alnylam Pharmaceuticals has submitted NDA 210922 seeking to market Onpattro "for the treatment of adults with hereditary transthyretin-mediated amyloidosis." A single clinical study is intended to provide substantial evidence of efficacy; ALN-TTR02-004 (APOLLO): a randomized, double-blind, placebo-controlled study of patients with Familial Amyloid Polyneuropathy (FAP). The primary endpoint was the difference between the patisiran-LNP and placebo groups in the change from baseline of mNIS+7 score at 18 months for the mITT population in subjects with hATTR-PN. Presence or absence of ATTR-cardiomyopathy (ATTR-CM) was not an inclusion criterion, and though some subjects had asymptomatic LV wall thickening, subjects with important cardiac symptoms were explicitly excluded from the trial (only NYHA FC I and II subjects could participate). Various echo parameters and NT-proBNP were measured and included as exploratory endpoints but not within a plan to spare alpha.

We observe the following:

- Study ALN-TTR02-004 does not provide any cardiac efficacy data. Imaging and serum biomarkers such as global longitudinal strain and NT-proBNP do not measure how a patient feels, functions, or survives, nor are they known to predict how a patient feels, functions, or survives and hence do not measure a clinical benefit.
- (b) (4) [redacted]
- If one is willing to accept the data selection process in study ALN-TTR02-004, the LV thickness changes were small, and the trend toward improvement in LV strain occurred after the apparent trend in improvement of LVEF (since strain is purported to be the more sensitive indicator of trends in LV systolic function, it should have changed first). The study ALN-TTR02-004 NT-proBNP data were skewed such that their analysis required modification. While that modification suggested a trend toward improvement, there were two open-label extension studies in which echocardiography and NT-proBNP were measured that showed no meaningful differences in these measures with 18 to 24 months of follow-up
- (b) (4) [redacted]

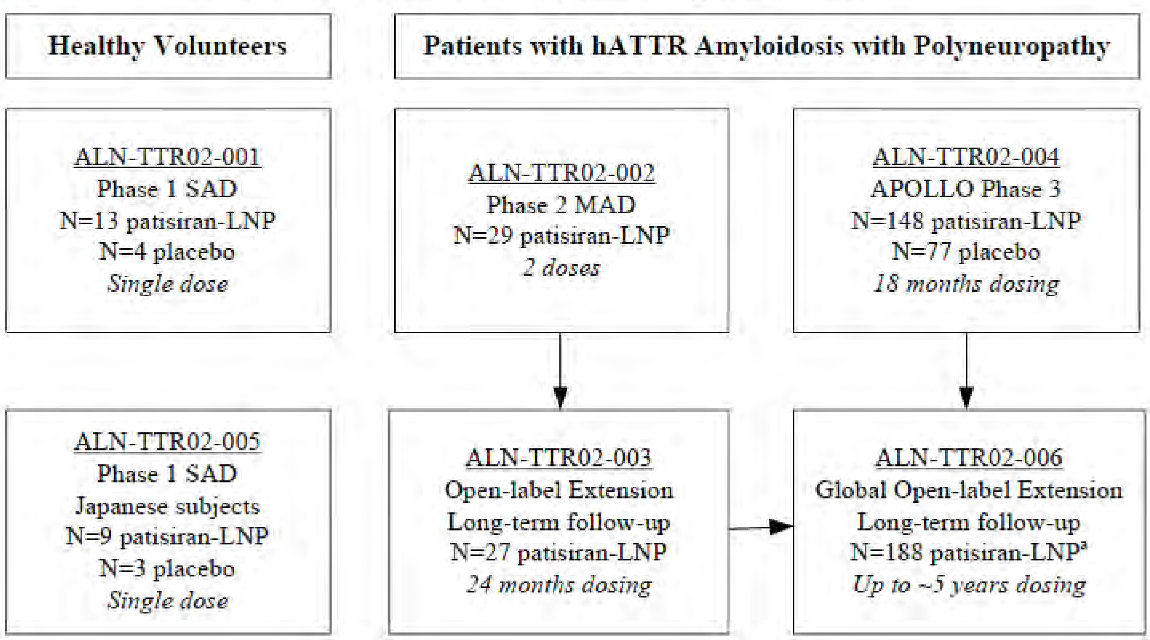
(b) (4)
[redacted]

We recommend:

- (b) (4) [redacted] we recommend that, if approved, the indication in the label should explicitly state that Onpattro is intended solely for treatment of Familial Amyloid Polyneuropathy.
- (b) (4) [redacted]

SCHEMATIC OF THE ONPATTRO DEVEOPMENT PROGRAM (sponsor):

Figure 1: Patisiran-LNP Clinical Development Program Schematic



SCHEDULE OF CARDIAC ASSESSMENTS ACROSS STUDIES:

Assessment	Brief Description	Interpretation of the Score	Schedule of Assessment by Study		
			004	003	006[a]
Cardiac assessments	Echocardiogram to evaluate cardiac structure (mean left ventricular [LV] wall thickness and LV mass), cardiac systolic function (longitudinal strain and left ventricular ejection fraction [LVEF]), and cardiac diastolic function (left ventricular end diastolic volume [LVEDV]). Cardiac biomarkers (NT-proBNP and troponin I) to evaluate cardiac stress and injury.	Less cardiac amyloid involvement = **Lower mean LV wall thickness or LV mass** Improved cardiac systolic function = **longitudinal strain further from 0 in the negative direction** (values increasingly abnormal as they approach 0); **Higher LVEF** Improved cardiac diastolic function = **increased LVEDV** (indicating a more distensible ventricle) Less cardiac stress = **Lower NT-proBNP** Less cardiac injury = **Lower troponin I**	Baseline, 9 and 18 months (all patients)	Baseline, 6, 12, 18, 24 months in the Cardiac Subpopulation only.	Baseline and at 12 months (all patients)

TRIAL ALN-TTR02-002: A PHASE 2, OPEN-LABEL, MULTI-DOSE, DOSE ESCALATION TRIAL TO EVALUATE THE SAFETY, TOLERABILITY, PHARMACOKINETICS, AND PHARMACODYNAMICS OF INTRA VENOUS INFUSIONS OF ALN-TTR02 IN PATIENTS WITH TTR AMYLOIDOSIS (POLYNEUROPATHY)

Design and CV Assessments

This was a multi-national, multi-center, Phase 2, open-label, multiple dose, dose escalation PK/PD study designed to determine the safety, tolerability, PK, and PD of 2 consecutive doses of patisiran in patients with ATTR. 29 subjects were enrolled in the study. Patients of any mutant TTR genotype with a biopsy-proven diagnosis of ATTR and exhibiting documented signs/symptoms of the disease (e.g., sensory, motor, or autonomic neuropathy) that were at least mild to moderate in severity were eligible for the study. Patients were required to be of stable cardiac status defined as NYHA class I or II subjects without unstable angina or uncontrolled clinically significant cardiac arrhythmias. The dose levels of patisiran evaluated in this study were 10, 50, 150, and 300 µg/kg Q4W or Q3W for two doses. Subjects were pre-treated with steroids/antihistamine/paracetamol to prevent infusion-related reactions (IRR). Echocardiography was obtained only at screening to rule out cardiac abnormalities in subjects without a normal ECG in the prior 90 days, and these were not read by a central laboratory. Serum troponins, CPK, CPK-MB were assessed on Days 1, 14, 42 and 56. Results of neither the baseline echocardiography nor the sequential CPK/CPK-MB or troponin results are mentioned in the CSR. There were no CV functional assessments performed. The were no CV endpoints.

TRIAL ALN-TTR02-003: A Phase 2, Multicenter, Open-Label, Extension Study to Evaluate the Long-Term Safety, Clinical Activity, and Pharmacokinetics of ALN-TTR02 in Patients with Familial Amyloidotic Polyneuropathy Who Have Previously Received ALN-TTR02

Dosing

All patients in Study ALN-TTR02-003 received the same dosing regimen of patisiran ([0.3 mg/kg intravenous (IV) q3w).] as this dose was demonstrated in study 002 to sustain >80% serum TTR suppression. In the event of an IRR, the infusion time could be extended to up to 3 hours.

CV Assessments

One of the stated tertiary objectives of this long-term safety study that enrolled 27 subjects rolled over from Trial ALN-TTR02-002 was to assess changes in cardiac structure/function through echocardiograms and serum levels of troponin I and NT-proBNP in patients with evidence of preexisting cardiac amyloid involvement. Patients qualified for the Cardiac Subgroup if baseline echocardiogram showed left ventricular wall thickness of ≥13 mm and there was no history of uncontrolled hypertension or aortic valve disease.

Cardiac function was assessed in all patients at Screening/Baseline with echocardiography. Patients who qualified to be in the Cardiac Subgroup underwent echocardiography every six months – these were analyzed by a central laboratory. NTproBNP and troponin I were assessed every 3 months.

No functional CV testing was performed (the 10-meter walk test, the test hand grip strength, and the FAP stage and PND score were performed as assessment of motor function).

Subject Disposition and Exposure

Per the sponsor, of the 29 patients who received patisiran in Study ALN-TTR02-002, 27 were enrolled in Study ALN-TTR02-003. Of the 2 patients who did not enroll in Study ALN-TTR02-003, one patient withdrew from ALN-TTR02-002 early due to AEs and one patient moved to a different country and was not interested in continuing. A patient was considered to have completed the study if the patient completed protocol specified procedures up through the 21-day Follow-up visit (Week 109 visit) for patients continuing treatment under a separate extension protocol, Study ALN-TTR02-006, and up through the 56-day Follow-up visit (Week 114 visit) for patients who chose not to continue patisiran treatment under Study ALN-TTR02-006. Two patients did not complete the study. One patient (Patient [redacted] (b) (4)) died of a myocardial infarction after completing all dosing but prior to the End of Study Visit and one patient (Patient [redacted] (b) (4)) withdrew from the study on Day 598 (after approximately 19 months on the study and receiving 27 doses of patisiran) due to an AE of [redacted]

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gastroesophageal cancer, which was subsequently fatal (after study discontinuation). Overall, patients were dosed with patisiran for a mean (SD) of 24.7 (0.21) months (range 19-25 months).

Results of CV Biomarkers and CV Imaging

The numbers of subjects in the Cardiac subgroup was small. There were no convincing improvements in either CV biomarkers (Troponin I or NT-proBNP) or echocardiographic parameters (LV wall thickness or average peak longitudinal strain) from Baseline to 24 Months, as sown in the following summary tables below (sponsor CSR):

Table 50: Summary of Serum Troponin I and NT-proBNP Mean Change from Baseline to 24 Months (2 Years), Cardiac Subgroup

Visit	Actual/ Change	Statistic	Troponin I (µg/L)	NT-proBNP (ng/L)
Baseline	Actual	N Mean (SEM) Median Min, Max	8 0.14 (0.08) 0.050 0.03, 0.69	9 809.8 (246.68) 604.0 105, 2070
Month 24	Actual	N Mean (SEM) Median Min, Max	10 0.06 (0.02) 0.030 0.03, 0.21	10 726.0 (244.63) 382.5 56, 2565
	Change from Baseline	N Mean (SEM) Median Min, Max	8 -0.09 (0.08) -0.01 -0.66, 0.03	8 -49.6 (170.83) -47.0 -986, 807

Table 52: Summary of Echocardiogram Results Change from Baseline to 24 Months (2 Years), Cardiac Subgroup

Visit	Actual/ Change	Statistic	EF	LV Wall Thickness	Average Peak Longitudinal Strain
Baseline	Actual	N Mean (SEM) Median Min, Max	11 62.46 (2.63) 63.33 40.71, 75.66	11 1.58 (0.06) 1.58 1.34, 1.92	11 -16.64 (1.32) -18.10 -23.0, -9.2
Month 24	Actual	N Mean (SEM) Median Min, Max	10 62.89 (3.66) 66.38 37.75, 76.45	10 1.47 (0.07) 1.42 1.13, 1.89	10 -16.53 (0.87) -17.05 -19.8, -11.8
	Change from Baseline	N Mean (SEM) Median Min, Max	10 0.63 (1.45) 0.73 -8.15, 6.84	10 -0.08 (0.05) -0.04 -0.41, 0.08	10 0.85 (0.89) 0.65 -4.1, 5.1

TRIAL ALN-TTR02-004: APOLLO: A Phase 3 Multicenter, Multinational, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of Patisiran (ALN-TTR02) in Transthyretin (TTR)-Mediated Polyneuropathy (Familial Amyloidotic Polyneuropathy-FAP)

Dosing

The primary objective of Study ALN-TTR02-004 was to determine the efficacy of patisiran-LNP (ALN-TTR02) by evaluating the difference between the patisiran-LNP and placebo groups in the change from baseline of mNIS+7 score at 18 months. Subjects in Study ALN-TTR02-004 (N=225) were randomized 2:1 to receive 0.3 mg/kg patisiran-LNP (or matching placebo) q3w. No individual dose adjustment was permitted. Excluded were subjects with type I diabetes, type II diabetes of more than 5 years' duration, New York Heart Association heart failure classification >2, ACS within the past 3 months, or uncontrolled arrhythmia or unstable angina. All subjects were pre-treated with a steroid, H1 blocker, H2 blocker, and paracetamol. The mITT Population included 148 patients in the patisiran-LNP group and 77 patients in the placebo group. The cardiac subpopulation was defined as those with a baseline LV wall thickness $\geq$ 1.3 cm by echo in the absence of aortic stenosis or hypertension by medical history.

CV Assessments

An exploratory endpoint was a comparative cardiac assessment defined by the change from baseline to Month 18 in LV wall thickness, LV mass, LVEF, LV longitudinal strain, NT-proBNP and troponin I, analyzed using the MMRM model, from the cardiac subpopulation (90 subjects in the patisiran-LNP group and 36 subjects in the placebo group). Change from baseline in 10-MWT gait speed was performed as a posthoc analysis. These same analyses were also performed on the mITT population.

Prespecified Echo Results (cardiac subpopulation)

Table 37: Change from Baseline in Echocardiogram Parameters Over Time (Cardiac Subpopulation)

			Mean LV Wall Thickness (cm)		LV Mass (g)		Longitudinal Strain (%)		Ejection Fraction (EF) (%)	
Visit	**Actual/ Change**	**Statistic[b]**	**Placebo (N=36)**	**Patisiran-LNP 0.3 mg/kg (N=90)**	**Placebo (N=36)**	**Patisiran-LNP 0.3 mg/kg (N=90)**	**Placebo (N=36)**	**Patisiran-LNP 0.3 mg/kg (N=90)**	**Placebo (N=36)**	**Patisiran-LNP 0.3 mg/kg (N=90)**
Baseline[a]	Actual	N	36	90	35	90	36	86	36	88
		Mean	1.639	1.682	264.52	275.48	-15.66	-15.13	62.21	60.00
		SD	0.2142	0.2573	77.709	80.109	3.513	3.410	8.607	9.918
		Median	1.615	1.640	243.67	270.94	-15.45	-15.10	62.99	60.64
		Min, Max	1.32, 2.22	1.31, 2.59	153.9, 433.8	155.8, 633.1	-23.6, -9.8	-23.4, -7.4	42.3, 75.7	33.4, 79.7
Month 18	Actual	N	25	79	25	78	25	79	24	79
		Mean	1.620	1.537	266.01	251.26	-14.12	-15.37	61.88	61.99
		SD	0.256	0.270	94.564	79.329	2.859	3.385	8.012	9.295
		Median	1.530	1.520	243.20	250.45	-13.80	-15.80	63.02	63.64
		Min, Max	1.32, 2.28	0.90, 2.27	132.8, 565.2	93.8, 582.4	-18.5, -8.4	-21.9, -8.3	46.3, 74.8	41.0, 76.0
	Change	N	25	79	24	78	25	75	24	77
		Mean	-0.018	-0.106	1.58	-16.14	1.41	0.04	0.46	1.04
		SEM	0.0328	0.0206	10.062	5.602	0.542	0.302	1.408	0.847
		Median	-0.020	-0.100	-0.51	-13.26	1.60	0.30	-0.21	0.20
		Min, Max	-0.39, 0.37	-0.69, 0.38	-80.8, 169.7	-205.4, 162.7	-5.2, 7.5	-6.8, 5.8	-11.8, 16.2	-18.1, 19.6
		LS Mean (SEM)	-0.007 (0.0332)	-0.100 (0.0195)	0.63 (9.427)	-15.12 (5.396)	1.46 (0.481)	0.08 (0.280)	0.57 (1.371)	1.00 (0.768)
		95% CI	-0.073, 0.059	-0.138, -0.061	-18.05, 19.31	-25.81, -4.42	0.50, 2.41	-0.47, 0.64	-2.15, 3.29	-0.53, 2.52
		LS Mean (SEM) Difference (Patisiran - Placebo)		-0.093 (0.0385)		-15.75 (10.862)		-1.37 (0.557)		0.43 (1.572)
		95% CI		-0.169 -0.017		-37.27, 5.78		-2.48, -0.27		-2.69, 3.55

Reviewer's comment: The point estimate for the placebo-corrected change from baseline LV Wall Thickness is reduced by less than one mm with the upper bound of the 95% CI being 1.7 mm. The LV mass trends downward on therapy, but it is important to note that LV mass is calculated from the LV wall thickness and LVEDD (so is not independent from the wall thickness assessment). These are changes that may exceed the totaled variabilities of the imaging modality (study reader, sonographer, and image angle). Interestingly, longitudinal strain is improved, driven by a more pronounced deterioration of the placebo subjects. LVEF was not changed. While the LVEF trend to improvement occurred in the first 9 months of follow-up, the LV strain improvement did not occur until months 9-18, which is not the physiologic pattern of what would be expected from a true

response (one would expect strain to improve before LVEF). This divergence is demonstrated in the following sponsor CSR figures:

Figure 24: Change from Baseline over Time in Longitudinal Strain, MMRM (Cardiac Subpopulation)

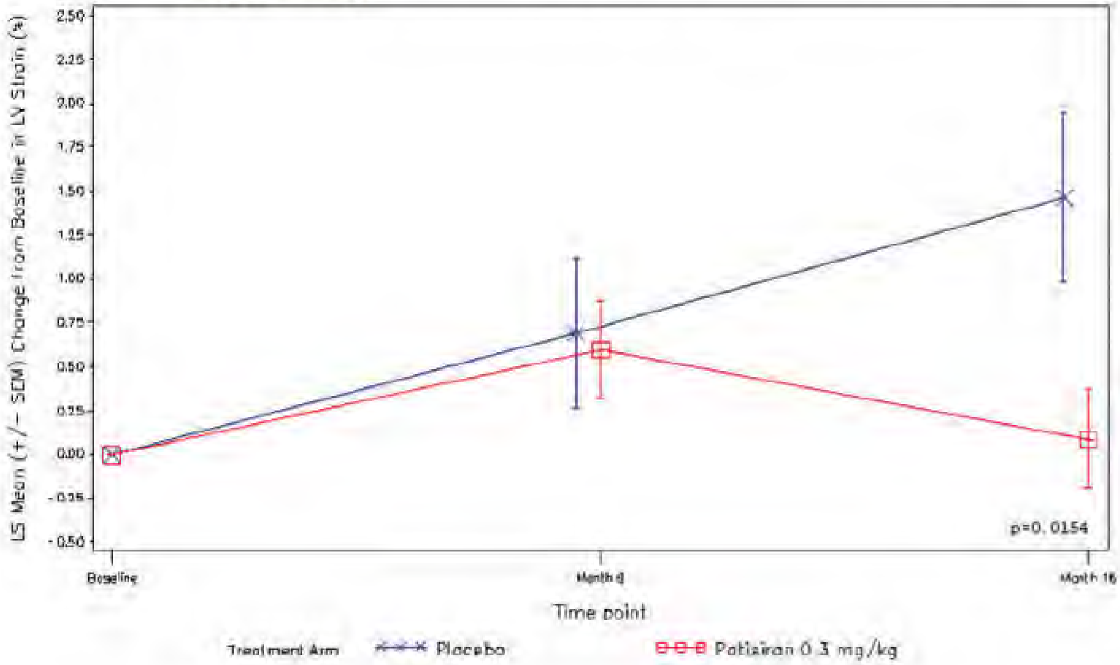
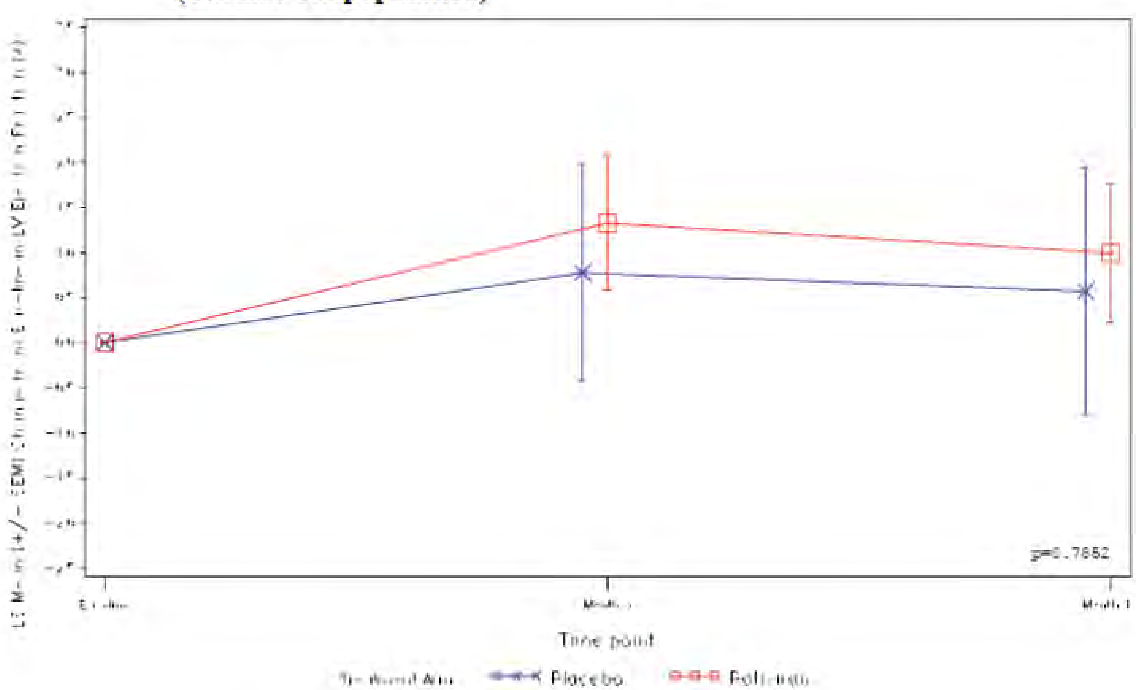


Figure 25: Change from Baseline over Time in LV Ejection Fraction (%), MMRM (Cardiac Subpopulation)



NT-pro-BNP (cardiac subpopulation)

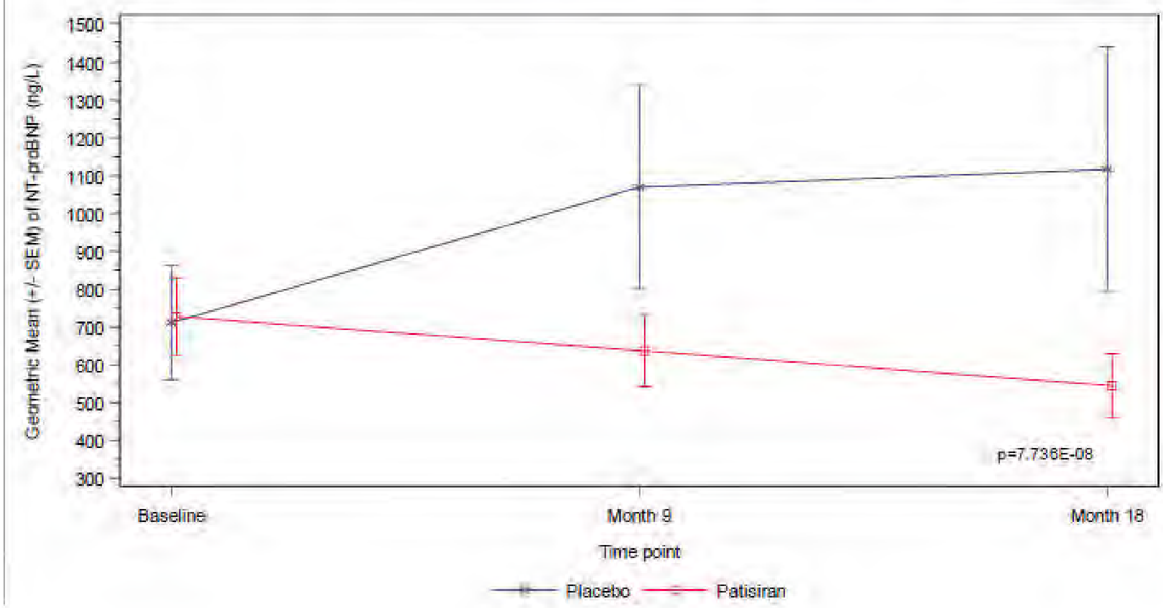
Table 38: Analysis of Mean Change from Baseline to Month 18 in NT-proBNP (ng/L), MMRM Model

			Cardiac Subpopulation		mITT Population	
Visit[a]	Actual/ Change	Statistic	Placebo (N=36)	Patisiran-LNP 0.3 mg/kg (N=90)	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Baseline	Actual	N	34	88	75	144
		Mean	1318.49	1512.35	1294.37	1246.68
		SD	1468.614	1754.036	2236.144	1787.982
		Median	845.74	756.44	562.81	474.48
		Min, Max	39.9, 6036.4	51.9, 7878.6	24.9, 16497.7	27.9, 9882.4
		Geometric Mean[b]	711.10	726.92	531.29	531.04
		SEM Geometric Mean[b]	151.079	103.015	86.661	59.618
		CV (%) Geometric Mean[b]	190.8	220.3	252.1	226.7
Month 18	Actual	N	24	80	53	137
		Mean	2942.76	1321.74	2184.03	1180.79
		SD	5748.01	1973.96	4136.79	2356.94
		Median	1208.46	626.71	918.09	365.26
		Min, Max	85.8, 28228.4	53.9, 12069.9	54.9, 28228.4	21.0, 20155.1
		Geometric Mean[b]	1116.75	544.09	844.40	417.10
		SEM Geometric Mean[b]	320.757	85.208	166.972	50.645
		CV (%) Geometric Mean[b]	249.8	247.3	263.5	255.7
Month 18	Change	N	23	78	52	134
		Mean	1888.68	55.85	1310.63	103.56
		SEM	985.039	149.442	460.159	118.889
		Median	320.35	-49.90	278.45	-32.94

Table 38: Analysis of Mean Change from Baseline to Month 18 in NT-proBNP (ng/L), MMRM Model

			Cardiac Subpopulation		mITT Population	
Visit[a]	Actual/ Change	Statistic	Placebo (N=36)	Patisiran-LNP 0.3 mg/kg (N=90)	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
		Min, Max	-467.0, 22191.9	-2286.3, 6871.7	-467.0, 22191.9	-2287.3, 10272.7
	Fold-Change to Baseline	Adjusted Geometric Mean Fold-Change[c]	1.97	0.89	1.91	0.90
		95% CI[c]	1.55, 2.50	0.78, 1.01	1.63, 2.23	0.81, 0.99
		Ratio of Adjusted Geometric Mean Fold-Change (Patisiran/Placebo)[c]		0.45		0.47
		95% CI[c]		0.34, 0.59		0.39, 0.56
		p-value[c]		7.736E-08		7.314E-14

Figure 26: Geometric Mean of NT-proBNP (ng/L) Over Time (Cardiac Subpopulation)



Reviewer's Comment: This is a posthoc modification of the NT-proBNP data in which was performed under the following rationale (sponsor CSR):

> *"NT-proBNP data was blinded during the study. After database lock, it was observed that the distribution of NT-proBNP data was highly skewed and consequently violated the assumption of normality for the MMRM model. Based on published literature, a logarithmic transformation was applied to normalize the distribution of NT-proBNP. Following natural log transformation (loge), an MMRM model was fitted, with the outcome variable as loge(postbaseline) - log_e(baseline) and fixed effect terms including log_e(baseline) as a continuous covariate, treatment arm, visit (Month 9 or Month 18), and treatment-by-visit interaction. Based on the MMRM model, the adjusted geometric mean fold-changes from baseline along with the 95% CI for each arm and the ratio of adjusted geometric mean fold-change (patisiran/placebo) and the corresponding 95% CIs were constructed by exponentially back-transforming LS means, difference in LS means, and the corresponding 95% CI. The nominal p-value for Month 18 was reported."*

The differential effect of this exploratory/hypothesis-generating analysis is intriguing in the setting of the trends in the 2-D echo data. It would be interesting to see these analyses performed on a population of ATTR-CM subjects with symptomatic cardiomyopathy.

Troponin I

The sponsor reports that, *"The majority of troponin I values (90.2%) were reported as <0.1 μg/L based on assay sensitivity, and all such values were imputed to 0.1 μg/L for analysis (Table 14.4.2.13). Accordingly, the lack of precision in troponin I data as collected prohibits an accurate assessment of patisiran-LNP treatment effect on troponin I."*

10-Meter Walk Test (posthoc, cardiac subpopulation)

The sponsor reports that, *"At 18 months, change from baseline in gait speed was +0.008 m/s in the patisiran-LNP group while the placebo group showed a change in gait speed of -0.346 m/s (LS mean difference between groups: 0.354 m/s, 95% CI: 0.242, 0.466). The improvement in gait speed in the patisiran-LNP group compared with placebo was evident at 9 months (LS mean difference between groups: 0.161 m/s, 95% CI: 0.076, 0.246) for patients in the patisiran-LNP group compared to the placebo group."* Findings for the mITT group were similar.

TRIAL ALN-TTR02-006: A Multicenter, Open-Label, Extension Study to Evaluate the Long-term Safety and Efficacy of Patisiran in Patients with Familial Amyloidotic Polyneuropathy Who Have Completed a Prior Clinical Study with Patisiran (study 003 and study 004)

Design and CV Assessments

Cardiac assessments included evaluation of echocardiogram, N-terminal prohormone of B-type natriuretic peptide (NT-proBNP), troponin I, and NYHA classification and were assessed in all patients. All patients enrolled in Study 006 are included in the analysis of cardiac assessments regardless of whether they met the cardiac subpopulation criteria in the parent study.

Echocardiography Data (change from baseline to week 52)

Table 24: Summary and Change from Baseline for Echocardiogram Results over Time

Parameter	Visit	Actual/ Change/ % Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
LV Wall Thickness (cm)	Baseline[a]	Actual	N	43	116	25
			Mean	1.534	1.489	1.249
			SD	0.2561	0.3131	0.3321
	Week 52	Actual	N	9	27	24
			Mean	1.437	1.353	1.210
			SD	0.3533	0.2889	0.3499
			SEM	0.1178	0.0556	0.0714
		Change	N	9	27	24
			Mean	-0.196	-0.185	-0.053
			SD	0.1142	0.1890	0.1622
			SEM	0.0381	0.0364	0.0331
LV Ejection Fraction (%)	Baseline[a]	Actual	N	43	115	25
			Mean	61.105	63.338	62.432
			SD	9.4731	8.2858	10.9976
	Week 52	Actual	N	9	23	24
			Mean	57.527	60.749	62.086
			SD	10.6413	10.1841	9.6605
			SEM	3.5471	2.1235	1.9719
		Change	N	9	23	24
			Mean	1.573	-2.127	0.185
			SD	13.4514	5.5946	8.8354
			SEM	4.4838	1.1666	1.8035
LV Strain (%)	Baseline[a]	Actual	N	43	115	25
			Mean	-15.85	-15.91	-17.72
			SD	3.328	3.299	4.079
	Week 52	Actual	N	9	27	24
			Mean	-14.07	-14.27	-16.05
			SD	2.250	3.954	5.510
			SEM	0.750	0.761	1.125
		Change	N	9	27	24
			Mean	1.09	1.63	1.37
			SD	2.951	3.330	4.597
			SEM	0.984	0.641	0.938

Table 24: Summary and Change from Baseline for Echocardiogram Results over Time

Parameter	Visit	Actual/ Change/ % Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
LV Mass (g)	Baseline[a]	Actual	N	43	116	25
			Mean	239.346	241.839	198.570
			SD	70.9539	84.4560	89.1780
	Week 52	Actual	N	9	27	24
			Mean	236.722	222.623	191.128
			SD	85.0454	78.6787	86.1409
			SEM	28.3485	15.1417	17.5834
		Change	N	9	27	24
			Mean	-39.341	-44.070	-11.750
			SD	37.3864	45.7663	23.3097
			SEM	12.4621	8.8077	4.7581

Reviewer's comment: LVEF drops in the active-to-active rollover group from study 004, with greater loss of LV strain in both active-to-active rollover groups at week 52. It seems incongruous that active-to-active rollovers from study 003 both improve their LVEF and drop their LV strain. This suggests variability occurring in a relatively low-resolution imaging technique.

~~NT-proBNP (change from baseline to Week 52)~~

Table 25: Summary of NT–proBNP over Time

Parameter	Visit	Actual/Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
NT-proBNP (pmol/L)	Baseline[a]	Actual	N	43	116	25
			Mean	206.682	126.653	33.329
			SD	240.0692	194.1345	49.8896
			SEM	36.6102	18.0249	9.9779
			Median	102.660	44.490	19.590
			Min, Max	6.61, 882.05	2.48, 1215.75	0.59, 224.32
	Week 52	Actual	N	10	28	24
			Mean	350.342	329.018	38.345
			SD	475.5034	1171.8688	50.9032
			SEM	150.3674	221.4624	10.3906
			Median	148.855	51.390	15.280
			Min, Max	6.73, 1334.46	3.07, 6280.31	0.59, 178.89
		Change	N	10	28	24
			Mean	9.557	180.439	5.191
			SD	262.1046	958.1113	21.4341
			SEM	82.8848	181.0660	4.3752
			Median	-1.420	-1.775	0.465
			Min, Max	-563.57, 452.41	-148.56, 5064.56	-57.70, 50.76

Reviewers Comment: The mean change from baseline in the placebo-to-active rollover arm appears anomalously small in this table. There are no clear trends. The NT-pro-BNP mean rose over the 52-month period in the placebo-to-active rollover sub-group from study 004.

Troponin I

The sponsor states that, *"there were no notable changes in serum troponin I levels over 52 weeks in any study group."*

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/s/

PRESTON M DUNNMON
03/17/2018

MARTIN ROSE
03/18/2018

NORMAN L STOCKBRIDGE
03/19/2018